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CLINICAL AND EXPERIMENTAL

THE EFFECT OF TEMPERATURE ON DIGITALIS ACTION*

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THE effect of the temperature on the action of digitalis is of practical importance. In sthenic fevers such as influenza it may be given with the idea of supporting and resting the heart. In acute infections there is still a difference of opinion about the value of digitalis therapy. For example, MacKenzie[1] says: "It is too often used when patients are dying from some grave affliction, as pneumonia. . . . I have never seen much good follow the administration of digitalis in acute febrile states." In such cases the action of digitalis on the heart may be complicated by the action of a toxin.

Ross A. Jamieson[2] studied the action of strophanthin on dogs and cats infected with pneumonia. He found no difference in the lethal dose in normal and infected animals. However, the temperature rise in his experiments was very slight, usually less than one degree.

Cohn and Jamieson[3] say that digitalis acts during the febrile period in pneumonia, and when there is auricular fibrillation or flutter, they believe it may be lifesaving.

Stone, Phillips, and Bliss[4] have corroborated the findings of Cohn and Jamieson in a clinical study in which 871 cases were observed.

Comparative Changes in Digitaloid Action Due to Temperature.—In the frog the fatal dose of digitalis injected into the lymph sac is about 600 mg. per

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kg., while in the cat it is about 100 mg., given intravenously. Much of this difference is undoubtedly due to difference in the temperature of the two animals.

Sollman, Mendenhall, and Stingel[5] have shown that between 10° and 15° C. the action of strophanthin in the frog is increased fourfold, and between 15° and 20° C. its toxicity is doubled. Analogous results were obtained by Baker and Haskell.[6]

Gunn[7] used isolated rabbits' hearts, and with variations of temperature, ranging from 28° C. to 41° C., found that with increase in temperature, strophanthin acts more rapidly.

Hirschfelder, Bicek, Kucera, and Hanson[8] studied the influence of high temperature on the toxicity of digitalis in cats. They found that when the temperature was raised by immersion in water heated to from 43° C. to 46° C. the toxicity was increased. The normal toxic dose, which was 0.94 c.c. of tincture per kg. at 39° C., fell progressively to 0.375 c.c. as the temperature rose to 43° C. At 41° C. the toxic dose was 83 per cent, at 42° C. it was 63 per cent, and at 43° C. it was about 40 per cent of the normal dose.

Hans Fischer,[9] working with frogs, found that an increase in temperature of 10° C. increased the toxic action of digitalis two or three times, due to hastening the fixation phase of the drug with the heart muscle. He concludes that the working of digitoxin is due to a chemical process.

To obtain a temperature rise we have used (1) dinitrophenol, and (2) diathermy. That dinitrophenol may introduce some complications we admit, but if there are such, we do not know them. Animals given larger doses of dinitrophenol die from the effects of hyperpyrexia, and, so far as we know, any complications introduced are due to the fever temperature, and are such as would follow from any rise in temperature. Similar results were obtained by both methods.

EXPERIMENTS WITH DIGITALIS

Dogs were anesthetized with 35 mg. per kg. of pentobarbital given intraperitoneally. Respiratory and blood pressure tracings were recorded. The temperature was read from a thermometer inserted through an incision in the abdomen into the peritoneal cavity. This method allows repeated readings of the thermometer without withdrawal. Dinitrophenol, 10 mg. per kg., was injected intravenously in a 1 or 2 per cent solution in distilled water. The dose of digitalis is given below.

Controls.—In a series of 21 dogs, using a tincture of digitalis prepared from Reference Digitalis Powder, U.S.P., we found that the average fatal dose was 1.2 c.c. per kg. when injected intravenously at the rate of 0.1 c.c. per kg. every five minutes. The variations in the fatal dose will range from 1 c.c. to 1.5 c.c., but the great majority were close to 1.2 c.c.. This method may be used with adequate accuracy to standardize digitalis. (A paper on this method of standardization will appear in the *Journal of Pharmacology and Experimental Therapeutics.*)

RESULTS WITH U.S.P. REFERENCE DIGITALIS (TINCTURE OF) IN DOGS

NO.	SIZE OF DOG (KG.)	FATAL DOSE MG. X KG.
1	11.0	120
2	9.5	110
3	16.8	105
4	9.3	139
5	7.2	136
6	9.5	120
7	9.5	134
8	9.5	100
9	5.6	125
10	12.5	125
11	11.0	120
12	7.3	100
13	15.5	150
14	6.4	145
15	15.7	130
16	7.5	125
17	7.3	125
18	7.9	116
19	10.0	110
20	11.4	106
21	5.2	140
	Average	123

With these results as a basis, we are able to study the effect of temperature on toxicity.

Digitalis Tends to Slow the Heart Rate at High Temperatures.—Digitalis slows the heart rate at increased temperatures, but with increasing temperature the heart muscle becomes more irritable, and the vagus control is greatly minimized. Digitalis tends also to slow the rate of temperature rise.

The following experiments show the effect of digitalis on the heart rate at the higher temperatures. Four dogs were used in this series. One of these experiments is given in detail. For the sake of brevity, the others are tabulated to show the comparative results.

DOG NO. 4—11.4 KG.

TIME (MINUTES)	TEMPERATURE	HEART RATE
00	38.9 (normal)	168 (normal
05	38.2	204 Given 10 mg. × kg. dinitrophenol
15	38.6	196 Given 1.1 c.c. Digitalis
20	39.0	184 Given 1.1 c.c. Digitalis
25	39.1	180 Given 1.1 c.c. Digitalis
30	39.7	180 Given 1.1 c.c. Digitalis
35	40.0	184 Given 1.1 c.c. Digitalis
40	40.3	172 Given 1.1 c.c. Digitalis
45	40.6	168 Given 1.1 c.c. Digitalis
50	40.8	156 Given 1.1 c.c. Digitalis
55	41.0	128 Given 1.1 c.c. Digitalis
60	41.2	fibrillating 1.1 c.c. Digitalis

Total temperature rise = 2.3° C.
Slowing of normal pulse rate = 40 beats per minute
Total dose of digitalis = 95 mg. × kg.

SUMMARY

DOG NO.	1	2	3	4
Weight (kg.)	16.8	17.2	4.5	11.4
Normal temperature	38.2	39.5	37.5	38.9
Normal heart rate	176	150	220	168
Total temperature rise	3.5	4.5	5.5	2.3
Total amount digitalis	80 mg. × kg.	50 mg. × kg.	70 mg. × kg.	95 mg. × kg.
Decrease in normal heart rate (per minute)	24	26	44	40
Average temperature rise	3.9°			
Average slowing of normal pulse	33			
Average digitalis dose	73 mg. × kg.			
Average heart rate after dinitrophenol, before digitalis				188
Average heart rate at low point following digitalis				134
Average decrease in rate per minute by digitalis after dinitrophenol				54

With increase in temperature, there is a definite increase in toxicity. The average rise in temperature is 3.25° C., and the toxic dose is 72.7 per cent of the normal fatal dose. The toxicity increase is approximately 10 per cent for each degree of rise in temperature. The individual variations are about the same in cases of high temperatures as it is in the normal animals. We have obtained similar results with diathermy, where the temperature was kept constant throughout the experiment.

TOXIC DOSE OF REFERENCE DIGITALIS (TINCTURE OF) WITH INCREASED TEMPERATURE DUE TO DINITROPHENOL—INJECTIONS OF DIGITALIS OF 0.1 C.C. PER KG. EVERY 5 MINUTES INTRAVENOUSLY

WEIGHT OF DOG KG.	NORMAL TEMPERATURE	TEMPERATURE AT WHICH DIGITALIS WAS GIVEN	AVERAGE	FATAL DOSE MG. × KG.	PERCENTAGE OF NORMAL FATAL DOSE
4.0	37.8	40.4-40.8	40.6	0.090	75
5.4	38.3	40.1-40.8	40.4	0.075	62
11.4	38.4	38.6-41.2	39.9	0.095	80
8.6	38.6	40.4-42.1	41.2	0.100	83
4.5	37.5	40.3-43.0	41.7	0.070	60
7.2	37.8	40.8-43.9	42.3	0.095	80
8.1	38.5	39.4-44.0	41.7	0.070	60
6.3	37.8	42.0-44.0	43.0	0.090	75
Average 7.0	38.1		41.35		72.7

Toxic Doses of Digitalis When the Temperature Is Raised by Diathermy.—In order to rule out the possibility that the results obtained using dinitrophenol were not due to a pure temperature effect, it was decided to try diathermy as a means of obtaining an increase in temperature. A high frequency diathermy apparatus made by Beck-Lee Company was used. The electrodes were placed over the anterior and posterior thoracic walls in such a way as to cover the entire thorax and upper abdomen. Temperature was recorded by introducing a thermometer into the abdominal cavity so that the bulb was in the area covered by the electrodes.

By this method it is possible to obtain a rapid rise in temperature. Variation of the current permits constant temperature readings throughout the experiment. In this way more exact values can be obtained for the dose of digitalis at various temperatures.

The average normal temperature of the dog is 38.6° C. Experiments were done at temperatures of 40°, 41°, 42° and 43° C. The same procedure for injection of digitalis was used as in the other experiments.

At 40° C. a total of 100 mg. per kg. of digitalis was found fatal, or 82 per cent of the normal fatal dose for dogs. As in the previous experiments, slowing of the heart was pronounced, the heart rate falling from 200 to 156.

At 41° C. the fatal dose was 81 per cent of normal. Slowing of the heart was again found, from 180 to 156.

At 43° C. the fatal dose was 61 per cent of normal. Heart rate fell from 176 to 148.

Summary

TEMPERATURE	FATAL DOSE (MG. × KG.)	PERCENTAGE OF NORMAL
40	101	82
41	100	81
42	80	66
43	77	61
44	60	50

These results confirm those obtained with dinitrophenol, and are in accord with the findings of Hirschfelder and others[8] in their work on cats. The method used has the advantage over dinitrophenol of permitting a constant temperature to be maintained for any desired period of time.

Effect of Diathermy Rise of Temperature on the Pulse Rate

TEMPERATURE C.		PULSE RATE		AFTER DIGITALIS
NORMAL	AFTER DIATHERMY	NORMAL	AFTER DIATHERMY	
38.6	40	200	204	156
38.9	41	144	180	156
37.8	42	196	212	116
37.8	43	148	176	148
38.6	44	168	240	198

The doses of dinitrophenol used in these experiments (10 mg. per kg. intravenously) killed by hyperpyrexia. About 45° C. is the highest temperature that dogs will stand. Some of them may die at a lower temperature, or about 43° C., if the temperature rises too rapidly. We cannot, therefore, be certain in all cases when digitalis is used at this high temperature that death was due to the digitalis. There seems no doubt, however, that the toxicity of digitalis is increased by temperature rise.

The use of dinitrophenol to raise temperature has one definite drawback, i.e., the temperature continues to rise throughout the experiment, so that it is practically impossible to state the degree of change in digitalis effect at a set temperature. Digitalis, however, seems to lessen the rate of temperature rise of dinitrophenol, and is to some degree antipyretic.

Action of Temperature on the Vagus and Sympathetics.—Increased temperature does not influence the function of the vagus, on the heart, as tested by

electrical stimulation, until the temperature reaches 43° C. or over (see Fig. 1), showing, therefore, that digitalis at increased temperatures under dinitrophenol might be effective in slowing the heart. We have found that at higher temperature digitalis does slow the heart rate to some extent.

The sympathetics, as judged by the response to adrenalin, are not increased in activity but are weakened by higher temperatures. At 43° C. the response to adrenalin is markedly lessened (see Fig. 2). These effects are what we might expect if we credit the "wisdom of the body" in avoiding a vicious circle. If increase in temperature lessened the effect of the vagus in slowing the heart, or increased the effect of the sympathetics in stimulating the rate, we would have a vicious circle due to the increase in temperature, and could expect little effect from digitalis. However, while digitalis may exert as great a percentage reduc-

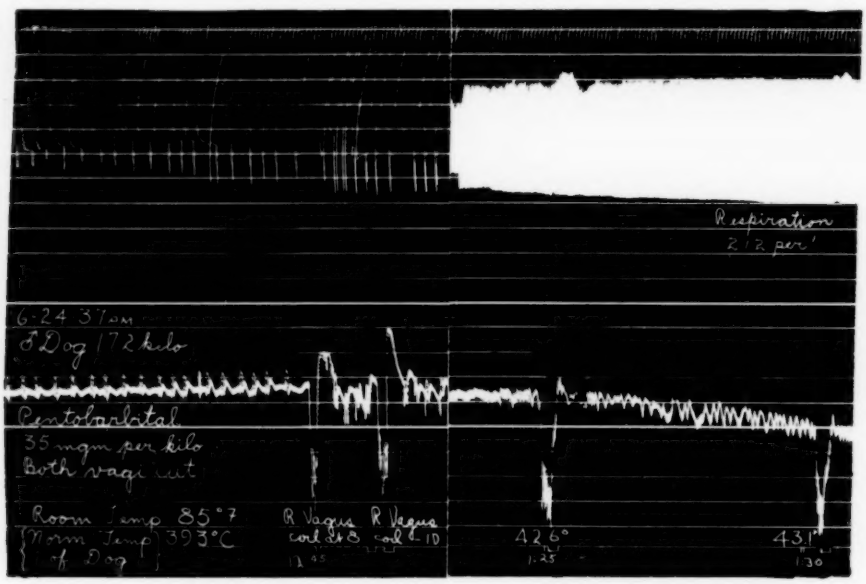

Fig. 1.

tion of heart rate at high temperatures, such a reduction might mean little where the fever temperature is high. An increase of 10 to 15 beats per minute in the heart rate for each degree of temperature rise would go far to obscure the slowing effect of digitalis. In addition the increase in toxicity of the digitalis at higher temperatures would lessen the difference between the therapeutic dose and the toxic dose (factor of safety). Perhaps the worst factor would be that where the lessened effect of digitalis in fever might suggest an increase in the dose. If this is done, there would be increased danger of toxicity, and this may be obscured by the rapid heart rate due to the temperature.

The fact that neither the vagus nor the sympathetics seem to be concerned in the rapid rate of the heart due to the rise in temperature by dinitrophenol leads to the conclusion that the irritability of the heart muscle is directly increased, due to the temperature. Since the toxicity of digitalis is exerted on

the heart muscle and mainly due to increased irritability, we may expect some additive toxic action at increased temperatures.

Effect of Temperature on the Heart Rate.—The heart rate increases progressively with the temperature. If no other disturbing factors, such as toxins, come into play, the increase is proportional to the rise in temperature on the average of about 15 beats for each degree rise. This effect seems due almost entirely to increased irritability of the heart muscle, for there is little, if any, effect on the function of the vagus or sympathetic nerves (see Figs. 1, 2, 3).

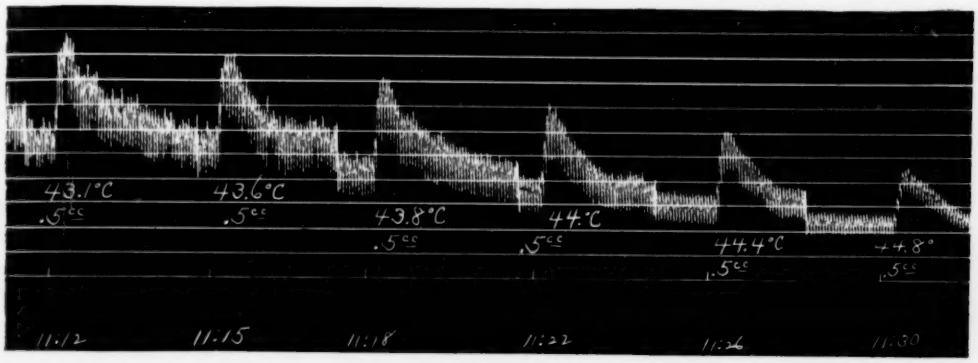

Fig. 2.

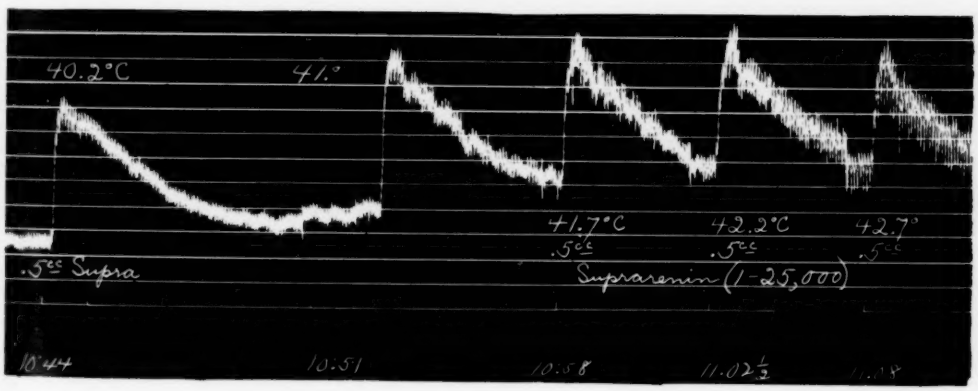

Fig. 3.

Figs. 2 and 3 are taken from a dog weighing 9.3 kg. In addition to the pentobarbital and dinitrophenol, the animal got 3 c.c. of 1 per cent morphine in the fourth ventricle. This accounts for the low blood pressure.

COMMENTS AND CONCLUSIONS

Digitalis at higher temperatures is somewhat more toxic. The increase in toxicity, however, is not enormous. With about one degree rise in temperature, Jamieson found no difference in the fatal dose of strophanthin for cats and dogs infected with pneumonia. Hirschfelder, working with cats, found that a rise of 2° C. effected an increase of about 17 per cent in toxicity, and for a rise of 4° C. toxicity increased at the rate of about 15 per cent for each degree rise. Our results on dogs show an average increase in toxicity of about 10 per cent for each degree rise. There is about the same individual variation in fever temperature as is shown at normal temperature.

There are good physiologic and pharmacologic reasons why we should not always expect benefit from digitalis in infectious fevers. Digitalis is not a direct beneficial agent. Any good it may do is in resting the heart and creating better conditions for its nourishment. There may be conditions where the increase in irritability caused by fever renders effective slowing difficult. This may occur in rising temperature and at highly elevated temperatures. Suitable conditions for nourishment must exist if benefit follows its use. In infection the condition of nourishment of the body is low; it may be on the negative side. Since digitalis per se adds no nourishment, its use may be without benefit. If, however, the fever is low and the toxic condition minimal, digitalis may be of benefit by resting the heart, and increasing its nutrition, especially where conditions indicate its use. If, however, the temperature is so high that the irritability of the heart muscle is greatly increased, digitalis is likely to be ineffective. This ineffectiveness may lead to the increased and dangerous repetition of dosage if the conditions and the action are not understood. These facts will explain why it may be beneficial in some febrile conditions and not in others. Such conditions will also explain the difference in opinion regarding the use of digitalis in fevers. A problem suggested by this work is the interaction of digitalis and antipyretics.

I wish to thank the Trustees of the U. S. Pharmacopoeia for furnishing Reference Digitalis Powder for this work.

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THE EXCRETION OF INGESTED ETHYL ALCOHOL IN SALIVA*

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THE concentration of ethyl alcohol in tissues and body fluids is now generally accepted as an index of the degree of intoxication. For determination of the latter, venous blood is usually analyzed. Various methods for obtaining other samples more conveniently have been proposed, all based upon the rapid diffusibility of ethyl alcohol through tissues. This property of alcohol is shown by the fact that within a short time after ingestion its presence can be detected in all tissues and body fluids.[2] Within one to two hours, that is at "equilibrium," the concentration is practically the same in all tissues and body fluids.[3] Nicloux[2] first demonstrated the excretion of alcohol in the saliva of dogs. Soon after initiation of the work reported in the present paper, Linde[4] demonstrated the equilibrium between plasma and parotid saliva in man. However, mixed salivary secretion was found by Linde to contain about 10 per cent less of alcohol than the parotid saliva. More recently Abels[5] has reported a close approximation between the alcohol concentration of venous blood and saliva.

In the present paper a simple and reliable procedure for the collection of mixed saliva will be described. It will be shown that, in addition to simple diffusion, other factors probably also affect the concentration of alcohol in saliva. However, the relatively slight differences which we found in the alcohol concentration of human blood and saliva do not prevent its application in physiologic work and for medicolegal purposes.

SAMPLING AND ANALYTICAL PROCEDURES

Collection of Saliva.—The mouth is rinsed thoroughly several times; the water is either swallowed or discarded after each rinsing. The subject should *wait at least five minutes before collecting the sample.* A small square of paraffin is then chewed. Flavored chewing gum should not be used. More consistent results are obtained if the first flow of saliva is discarded. Five cubic centimeters, or more if possible, are collected in a small test tube (18 by 200 mm.) which is provided with a tightly fitting stopper. The tube should contain approximately 10 mg. of sodium fluoride to preserve the sample. For best results the sample should be kept in the refrigerator and all analyses

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should be made within twenty-four hours. For collection of subsequent samples it is not necessary for the subject to rinse the mouth, unless alcohol has been taken in the intervening period.

The only precaution necessary is a thorough removal of the remaining traces of ingested alcohol from the mouth. The error from this source is shown by the following experiment with three subjects who had not ingested alcohol during the preceding forty-eight hours. A 20 per cent solution of alcohol was held sixty seconds in the mouth and then discarded. The mouth was not subsequently rinsed. After ten minutes, each subject was given a cube of paraffin and a sample of saliva was collected. These samples from the three subjects contained 140+, 38, and 26 mg. of alcohol per 100 c.c. Although 99 per cent or more of the initial 20,000 mg. thus disappeared within the ten-minute period, enough remained to make the calculation of alcohol excretion impossible. Another set of samples collected after thirty minutes contained no measurable alcohol. Ingested alcohol is thus rapidly removed from the mouth, due no doubt to rapid diffusion into the tissues and to the heavy flow of saliva induced by its presence.

Determination of Alcohol.—One cubic centimeter of saliva was transferred to a 300 c.c. Kjeldahl flask. The pipette was allowed to empty slowly, since saliva is viscous. The distillation and final oxidation were then carried out by the method of Friedemann and Klaas.[6] One cubic centimeter samples of blood and urine were similarly analyzed.

EXPERIMENTAL

Four experiments were performed on dogs, and 9 with human subjects. Since the experiments with dogs gave similar results, only 2 (see Figs. 1 and 2) are reported. Three of the human subjects received alcohol in a single small dose. The data shown in Fig. 3 are typical of the results obtained from these 3 subjects. Four subjects received a moderately large dose of alcohol. The results from three subjects are shown in Table I. None of the 7 subjects had received food within eighteen hours preceding the experiment. Two of

TABLE I

ALCOHOL CONTENT OF VENOUS WHOLE BLOOD, SALIVA, AND URINE OF HUMAN SUBJECTS AFTER INGESTION OF ETHYL ALCOHOL

Each subject received a 20 per cent solution of alcohol equivalent to 1 c.c. of 95 per cent alcohol per kg. of body weight. Urine collections were at one-half hour intervals in the case of subjects K and M. Subject L collected a sample at the end of each hour.

	SUBJECT K			SUBJECT L			SUBJECT M		
HOURS	VENOUS WHOLE BLOOD	SALIVA	URINE	VENOUS WHOLE BLOOD	SALIVA	URINE	VENOUS WHOLE BLOOD	SALIVA	URINE
0.5	52	72	25	125	145		65	121	27
1.0	97	102	129	101	111	114	96	126	132
1.5	99	109	139	89	92		94	111	127
2.0	98	104	141	82	87	117	87	101	115
2.5	94	100	134		76		81	95	103
3.0	87	84	117	65	69	87			

the human subjects received larger quantities of alcohol taken in several doses in the course of thirty to ninety minutes; these subjects had previously ingested food. The results are shown in Figs. 4 and 5.

The results from Dog 1 are shown in Fig. 1. The animal received no food during the preceding twenty-four hours. Pentobarbital sodium anesthetic was used. The submaxillary duct was cannulated, and the flow of saliva was increased by *chorda tympani* stimulation. Venous blood was obtained from a side branch of the jugular vein, arterial blood from the carotid artery, and urine from the cannulated ureter. The animal, which weighed 17 kg., received 100 c.c. of a 7 per cent (by weight) solution of alcohol by stomach tube. Samples were collected at frequent intervals as shown by the chart. The collection of saliva required from three to five minutes; the results are represented by solid bars of varying lengths. Venous blood is represented by solid

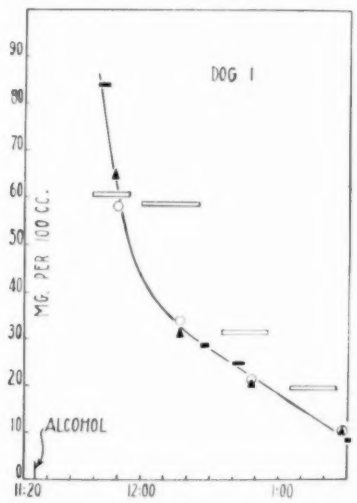

Fig. 1.

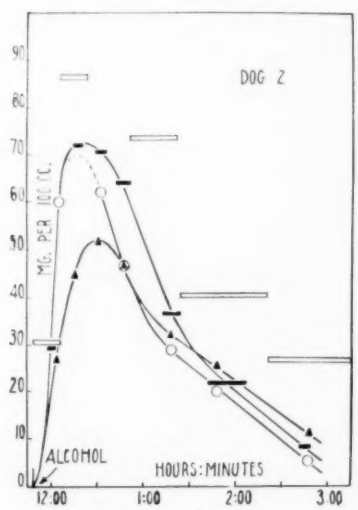

Fig. 2.

Fig. 1.—The concentration of alcohol in arterial and venous blood, saliva, and urine of Dog 1. In this and subsequent figures the alcohol content is represented as follows: arterial blood, open circles; venous blood, solid triangles; saliva, solid rectangles; urine, open rectangles.

Fig. 2.—Dog 2. See Fig. 1 for explanation of points.

triangles; arterial blood by open circles, and urine by open bars of varying length. The absorption of alcohol was unusually rapid in this animal. Instead of a calculated maximum of about 33 mg. per cent, the concentration no doubt was well over 90 mg. per cent. This maximum was probably reached within fifteen minutes. In this animal also the equilibrium, that is an equal distribution by diffusion into the tissues, was attained with unusual rapidity. This is shown by the fact that the three sets of results from blood and saliva agree closely. It should be noted that the concentration of alcohol in the saliva was slightly higher than in the venous blood and slightly lower than in arterial blood. It appears to represent an average between the arterial and venous concentrations. However, no such relation can be seen in the results from urine.

The experimental conditions were almost identical in the case of Dog 2. However, the flow of saliva was stimulated by means of pilocarpine. The results, which are shown in Fig. 2, appear to differ considerably from those of Dog 1. The rate of absorption of alcohol was not as rapid. The maximum concentration in urine and saliva (perhaps also in arterial blood) was obtained in about thirty minutes; the maximum in the venous blood was reached in about forty-five minutes. The three sets of data differed widely. While the concentration in saliva may have closely paralleled or even equalled that of the arterial blood during the first thirty minutes, it thereafter differed considerably from and remained higher than the arterial blood throughout the remainder of the experiment. To be noted is the arterial concentration which in the later stage was lower than the venous concentration. Again, the alcohol content of saliva in the later stage was approximately equal to the average

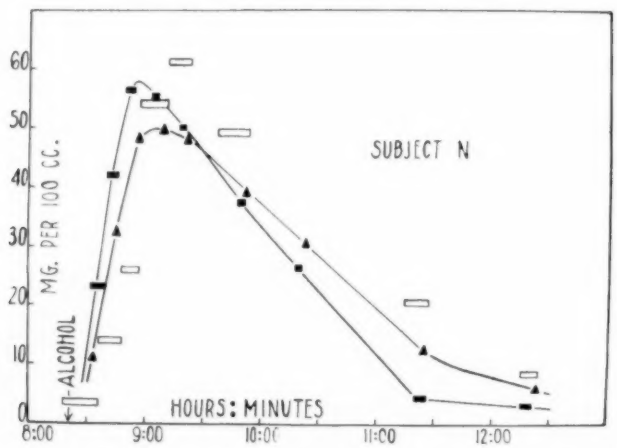

Fig. 3.—Human subject. The concentration of alcohol in saliva (solid rectangles) and urine (open rectangles), as compared with venous blood (solid triangles) is shown. Alcohol was ingested in a single small dose.

of the arterial and venous concentrations. Again, also, the alcohol content of urine throughout was higher than saliva or blood, and while the results were parallel, they did not approximate the results from blood, as was the case with saliva.

The curves shown in Fig. 3, which are typical of the results from 3 human subjects, represent the alcohol concentrations in saliva and venous blood after a single dose of alcohol. The latter consisted of 300 c.c. of 7 per cent ethyl alcohol which was ingested in the course of a few minutes. The subjects had received no food in the preceding eighteen hours. In two of the subjects the alcohol concentration in the saliva increased more rapidly than in the venous blood. From one subject, a habitual drinker, identical results were obtained during the initial stage of rapid increase. The saliva maximum, as in Fig. 3, was usually attained somewhat sooner than the venous maximum. The saliva concentration then declined more rapidly than the venous concentration and, in the later stage, either continued slightly higher or became slightly lower

than the venous concentration. The latter was the case in 3 subjects (see Figs. 3, 4, and 5). In several respects our curves thus differ from those given by Linde.

Fig. 4 represents the results from an experienced male drinker, weighing 74 kg. The subject had received food four hours previously. The alcohol, 150 c.c. of whiskey, was given in two doses. The absorption was relatively slow, and the difference between the alcohol concentration of saliva and venous blood was not as great relatively as in Fig. 3.

The results shown in Fig. 5 were obtained from a male subject who weighed 64 kg. and who received 285 c.c. of whiskey. The latter was ingested in the course of ninety minutes. Food had been taken one hour previously. This subject became profoundly nauseated and was markedly intoxicated. However, he could still cooperate in the collection of samples. Ingestion of the alcohol was begun at 1 P.M. At 2:40 and 3:05 the saliva concentrations were 206 and 216 mg. per cent, respectively. The subject was then placed on

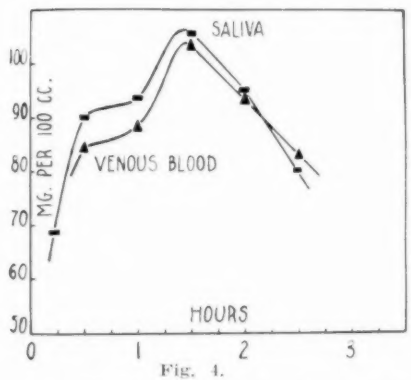

Fig. 4.

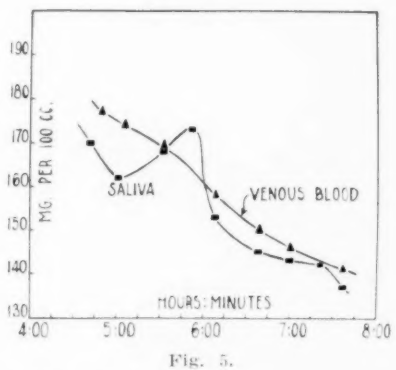

Fig. 5.

Fig. 4.—Human subject. Alcohol was ingested in two large doses.
Fig. 5.—Human subject.

a divan and allowed to sleep until 4:45. He then sat in a chair from 5:00 until the end of the experiment. At about this point the venous concentration, which had been falling, increased slightly. On the other hand, a marked increase which continued for almost one hour was noted in the saliva. The concentration of alcohol in the saliva then rapidly declined and again became slightly lower than in the venous blood. This experiment is presented to illustrate a number of points. First, the maximum concentration of alcohol attained in this experiment represents perhaps the upper limit at which intelligent cooperation of the subject may be expected. Within the range of concentrations shown in Fig. 5 most individuals became markedly intoxicated. Second, marked variations may be observed in the alcohol content of the saliva with only slight variations in the venous blood. The probable causes of these variations will be discussed below. Finally, the apparently large difference, after equilibrium, of 13 mg. per cent between the alcohol content of the saliva and venous blood noted at 5:00 represents only 7 per cent of the alcohol of the venous blood.

While the analytical data from mixed saliva thus show variations from those of blood, even more marked variations are noted in the case of uncatheterized urine specimens. The value of results from catheterized urine is unquestioned. Haggard and Greenberg[7] have shown that the alcohol concentrations of arterial blood and urine obtained by catheter are identical, provided allowance is made for the difference in water content of the two fluids. However, in most instances, the use of the catheter for urine collection is out of the question. It is necessary, then, to collect samples over short periods of time; but this too may prove difficult with a slow rate of excretion. Furthermore, the result from urine so collected does not accurately represent the concentration at the time of collection. This is illustrated by the results shown in Fig. 3. The alcohol content of urine in this subject during the period of rapid absorption increased at an apparently slower rate than of venous blood. On the other hand, the alcohol content of saliva was higher than that of venous blood during this stage, which is in keeping with the view that the concentration of alcohol in saliva approaches the concentration in arterial blood during the first stage (see Figs. 1 and 2). Further results illustrating the rate of excretion of alcohol in saliva and urine, as compared with venous blood, are shown in Table I. In the case of subjects K and M, urine samples were collected at one-half hour intervals; subject L collected urine at one hour intervals. The first one-half hour urine samples contained less alcohol than the blood; saliva contained considerably more of alcohol, as also in the case of subject N shown in Fig. 3. Subsequent samples of urine, collected at 1.0, 1.5, 2.0 and 2.5 hours, contained from 14 to 41 per cent, with an average of 32 per cent, more of alcohol than the venous blood. Saliva, simultaneously collected, contained from 2 to 31 per cent, with an average of 11 per cent, more of alcohol than the venous blood.

DISCUSSION AND CONCLUSIONS

Throughout this investigation we were interested chiefly in two questions: First, does simple diffusion alone account for the alcohol of mixed saliva, or is the secretion influenced by other factors? Second, is the estimation of alcohol in mixed saliva applicable for medicolegal purposes?

The concentration of alcohol in body fluids and excretions is best compared when the results are calculated on the basis of the water content. Thus, at equilibrium by diffusion, saliva should contain about 1.20 times as much alcohol as whole blood. On the same basis this ratio should also apply to urine. By such calculations Widmark[3] demonstrated the equilibrium of alcohol by diffusion between venous blood and urine one to two hours after ingestion of the alcohol. The alcohol content of urine is, however, more closely related to the arterial blood, as was shown by Haggard and Greenberg.[7] By considering only the data in the descending portion of the curve, Linde found an average of 1.21 times as much alcohol in parotid saliva as in venous blood. However, mixed saliva was found to contain only 90 per cent as much alcohol as parotid saliva. On the basis of our experience, this difference is larger than the error from volatilization during collection of the sample. It indi-

cates, therefore, that some of the salivary secretions contain considerably less alcohol; that other factors besides simple diffusion modify the secretion. It should be pointed out that many substances which are freely diffusible through the kidney glomeruli, such as urea, creatinine, and glucose, or constituents such as lactic acid and glucose,* which are found in many tissues in concentrations approximately equal to that in blood, appear in relatively smaller quantity in the saliva. Normal saliva, for example, contains only from 5 to 10 mg. per cent of glucose. A smaller concentration in the saliva, due to the *selective action* of the salivary cells, therefore, should not be unexpected or considered unusual. Furthermore, since the ingestion of alcohol produces peripheral vasodilatation and splanchnic vasoconstriction, a marked effect on the blood flow through the salivary glands is to be expected. The method of stimulation and changes in the rate of blood flow have long been known to affect the composition of saliva. It is not surprising, therefore, to find that our results indicate variations in concentration which are particularly marked during the period of rapid absorption and immediately following. The results shown in Figs. 2 and 5 are greater than can be accounted for by diffusion or by a marked increase in arterial alcohol concentration, and suggest that under unusual circumstances alcohol may be *excreted*. On the whole, however, these two effects appeared to be of minor importance, especially in the experiments with human subjects. This conclusion is based upon the observation on dogs that the maximum concentration in saliva coincided with the maximum in arterial blood. This maximum is reached before the maximum in the venous blood, unless the absorption is slow as in Fig. 4. The concentration in saliva parallels the arterial concentration and rarely is higher than can be accounted for by simple diffusion. During the later stage, although the saliva concentration as a rule is slightly lower than the venous concentration, the results agree within 10 per cent. During this stage we can assume that alcohol returns from tissues into the venous blood. The presence of alcohol in mixed human saliva can, therefore, be attributed chiefly to simple diffusion.

On the basis of these experiments we would recommend the analysis of mixed saliva as a convenient and fairly accurate measure of the alcohol content of human blood. The collection of saliva, it should be noted, requires no previous training of either the operator or the subject. It does, however, require the cooperation of the subject. The sample is small and is quickly collected; it can be obtained more frequently and is more representative of the period than a sample of urine. It is thus of particular value in physiologic experiments. Loss from volatilization is minimized by collecting the sample in a test tube, rather than on a watch glass.[4] It requires no special apparatus or sterile technique, nor does it produce discomfort as in the collection of blood. Unlike the collection of urine, the sample can be taken at any time or place without embarrassment to the subject. The analysis of saliva presents fewer analytical difficulties than the analysis of either blood or urine.[6] Because of its low organic matter content, very little foaming is en-

*Note that each of these substances contains several reactive groups, such as hydroxyl, amino, aldehyde, and carboxyl. Each of these substances also readily forms salts and ionizes, while ethyl alcohol is relatively inert in this respect.

countered in the distillation, and the titration in the case of normal saliva is practically equal to that of the water blank. It is even possible to precipitate practically all of the interfering substances (by means of $CuSO_4$, $HgSO_4$, $Fe_2(SO_4)_3$ reagent, followed by $Ca(OH)_2$ suspension) and to determine the alcohol in the supernatant solution by direct oxidation without previous distillation.[1] The difference between the alcohol concentration of mixed saliva and blood is relatively slight and, for medicolegal purposes, is not significant.

SUMMARY

A procedure is described for the collection of human mixed saliva.

The excretion of ingested alcohol has been determined in urine, saliva, and venous and arterial blood of man and dog.

In human subjects the alcohol concentration of mixed saliva more closely approaches that of venous blood than does urine. For this and for technical reasons the use of saliva is proposed for medicolegal purposes.

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A COMPARISON OF METHODS FOR DETECTING AND GRADING SUBCLINICAL SCURVY*

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THE development of our conception of subclinical scurvy has stimulated dozens of reports on methods of detecting mild forms of vitamin C deprivation. The first studies were based on measurements of capillary fragility.[1, 2] The tenor of these early reports was that, judging by capillary fragility, a condition of subclinical scurvy was extremely common in civilized communities.

These pioneer reports appeared the same year that the chemical nature and isolation of vitamin C were announced. Subsequently all the original claims have been re-examined by the newer chemical methods, usually based on measurement of vitamin C excretion or concentration in the blood. But while the newer studies have confirmed the original reports that subclinical scurvy is extremely common,[3-5] they have disagreed in the methods for demonstrating the condition. Accordingly, we find reports criticizing each of the proposed methods.

The present work is an endeavor, based on intensive examination by all methods, of sixteen scorbutic, two nonscorbutic patients with bleeding, and five normal persons, to determine the advantages and disadvantages of each test and to determine the best procedure available at this time.

The report is a further development of capillary resistance studies previously reported by this laboratory.[2, 8] During the earlier work, information regarding the relation to blood and urine vitamin C was necessarily lacking. It was also realized that variables due to unknown causes were present which would require further study. The present report is intended to define these limitations more precisely.

METHODS

Clinical.—The bedside diagnosis of subclinical scurvy is difficult. Symptoms and signs are vague and are frequently cloaked by the manifestations of a more conspicuous complicating disease. The most helpful criterion is undoubtedly a history of, or the presence of bleeding. Bleeding gums and epistaxis are the commonest but gastrointestinal hemorrhages, menorrhagia, subdural hematoma and petechial hemorrhages appearing during the course of venepuncture have all occurred in our own experience and have been the only clinical sign of vitamin C starvation. To such criteria may be added the dietary history, usually too indefinite to afford much support to a diagnosis.

*From the Laboratories of Grasslands Hospital, Valhalla.
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Capillary Resistance.—The method used throughout this study is a modification of that originated by Hecht.[2] Negative pressure is applied through the medium of a 1 cm. glass cup to the outer aspect of the arm. Pressure is maintained by use of a hand pump for one minute, and the resistance is expressed as the least negative pressure required to produce macroscopic petechiae. No attempt is made to read closer than 5 cm. of mercury negative pressure. All readings are made with the naked eye without cover glass pressure. Slight diffusion of the separate bleeding points is present in a positive

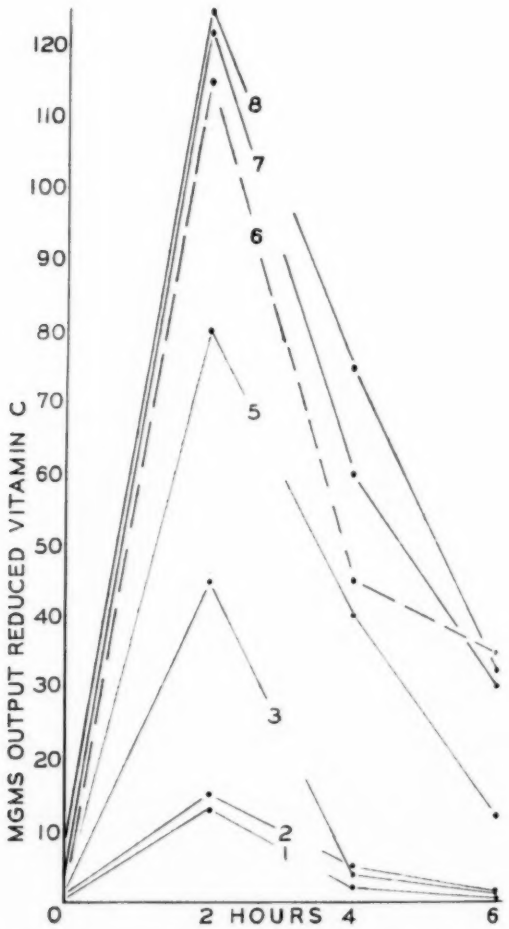

Fig. 1.—Urinary excretion curves. The curves represent the excretion during six hours on each of 8 daily, consecutive determinations. Normal values are indicated by the broken line. Three and one-half grams of vitamin C were required to saturate this patient (No. 15), a case of chronic peptic ulcer with hemorrhage. The numerical values were as follows:

Day	Per cent excreted in six hours	Intake/output ratio	Intake (mg.)
1	4	28	1,000
2	6	17	500
3	10	10	500
4	—	—	500
5	27	4	500
6	40	2.5	500
7	42	2.4	500
8	48	2.1	500

test. Interfering factors which may mar the efficiency of the test, especially in the milder deficiencies, are thickness of skin, position of the arm (passive congestion), and anemia (Figs. 5 and 6). Thirty centimeters of mercury negative pressure is considered as the lower limit of normal.

A modification of this method has also been used. This consists of raising the pressure to 45 cm. of mercury with the idea of causing a definite hemorrhagic area. Readings are taken immediately preceding injection of a test

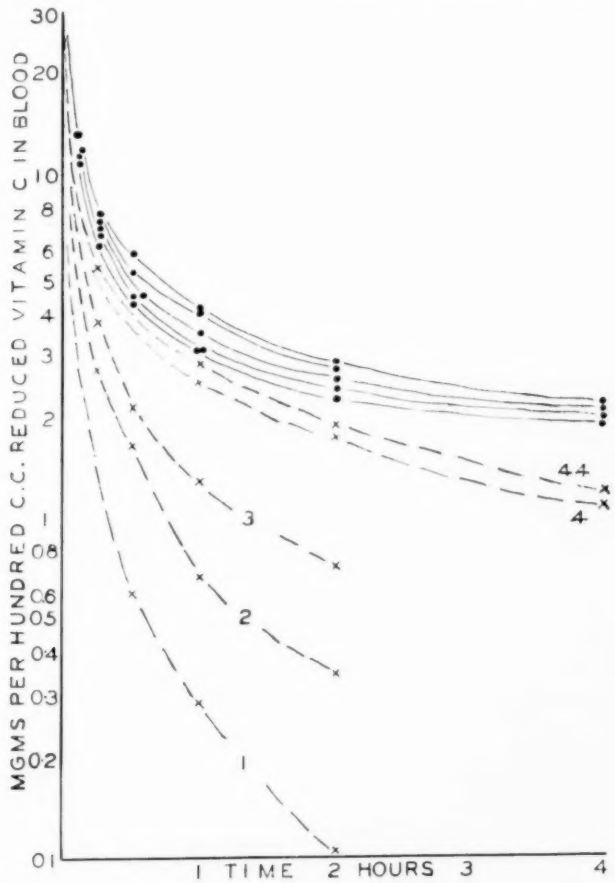

Fig. 2.—Blood absorption curves. The results of single determinations of the responses of 5 normal individuals are shown in solid lines. The broken lines represent the value secured in a case of lymphogranulomatosis in whom repeated determinations were made on the first, second, third, fourth, and forty-fourth days following treatment with vitamin C. Between the fourth and forty-fourth days the patient received 250 c.c. of orange juice daily.

dose of vitamin and then repeated at variable intervals of time after injection. Five minutes was the usual time interval in this study. Comparison between the amount of hemorrhage at the respective five-minute intervals as compared with the preinjection test was found a useful means of judging the patient's response to intravenous vitamin C.

Urinary Vitamin C.—Regardless of the type of analysis all urine specimens were handled in the same way. Immediately following voiding, glacial acetic acid was added in the proportion of one part of acid to ten parts of

urine and immediately sent to the laboratory. Titrations were carried out with 2:6 dichlorophenolindophenol (La Motte). Repeated titration of the dye with an ascorbic acid standard revealed that, in an original concentration of 0.097 mg. of vitamin C per c.c., the dye would lose 0.001 mg. of ascorbic acid value daily. With this allowance the dye was made fresh weekly, the new and old dye being titrated at the beginning and end of the week.

Titration of fresh, acidified, fasting urine has been used as a case finding procedure and prior to all intravenous study. One and one-half milligrams per cent is considered the lower limit of normal.

Twenty-four-hour specimens were followed on many of the patients before starting intravenous medication and as a means of following excretion during oral administration. Collections were made over a period of twenty-four hours, starting at 7:00 A.M. At each voiding the urine was acidified with glacial acetic acid and stored in a dark bottle on ice.

Six Hour Excretion Following Test Dose.—The six hour urinary excretion test was devised as a more rapid means of determining the vitamin C status of out-patients. This test is an abbreviation of the excretion test used by Harris and Ray.[4]

The test was started at 8:00 A.M. The patient was allowed to empty his bladder of night urine about 6:00 A.M. and the accumulation during the next two hours collected as the fasting specimen. Immediately prior to injecting the vitamin a capillary resistance test was performed, and all the arrangements made for doing a urinary excretion, constant pressure capillary resistance test, and blood absorption curve simultaneously. During earlier cases a 100 mg. test dose was used, but this was discarded in favor of a dose proportional to body weight. The amount was arbitrarily set at 15 mg. per kg., after experience with doses as high as 1,500 mg. Vitamin C in the form of "Cebione" (synthetic) supplied through the courtesy of Merck and Company, was used throughout the study. The dose calculated on the basis of 15 mg. per kg. was dissolved in 15 to 20 c.c. of distilled water. The injections were given slowly. This last precaution is still adhered to, but seems unnecessary. In order to evaluate the effect of rapid injection, the rate was gradually increased in a few patients receiving daily injections. At no time during the study was any sign of reaction noted regardless of the speed of injection.

During the latter portion of the study blood absorption curves were run in conjunction with the urinary excretion and capillary resistance constant pressure tests. When this was the case, a fasting blood sample was taken, the syringe removed, and a 20 c.c. syringe, containing the calculated dose, was applied to the same needle. Urine specimens were collected at two-hour intervals. Because of early confusing results due to a failure of the patient to void at the specified times, it was decided to give 250 c.c. of water at the time of injection and again following the collection of the first two-hour specimen to allow ample fluid for proper kidney function. In cases that failed to take water during the test or for a twelve-hour period prior to the test, a wide divergence was noted in the blood absorption and urinary excretion curves. This difficulty was minimized by supplying water.

Results of the six hour urinary excretion tests have been expressed in a variety of ways. The six hour intake-output ratio (6 hour $\frac{I}{O}$) is arrived at by dividing the intake by the total six hour excretion. A ratio below 2.5 was found compatible with normal saturation. A similar result is expressed in percentage as used by other workers.[5]

Blood Vitamin C.—Five cubic centimeters of venous blood was placed in a small slender tube, containing two drops of 20 per cent potassium oxalate to prevent clotting. Preparation of the filtrate was started promptly after venepuncture. In no case was more than twenty minutes allowed to elapse before initial centrifugation. Following separation of the cells and plasma, 2.1 c.c. of the latter were thoroughly mixed with 1.9 c.c. of 20 per cent tri-

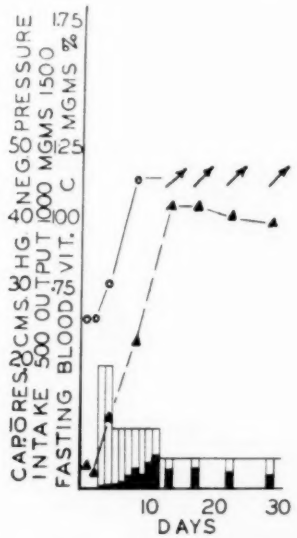

Fig. 3.—Rate of recovery of normal blood concentration of vitamin C, capillary resistance, and urinary excretion of vitamin following oral administration of 400 mg. of vitamin C as orange juice. Also showing maintenance of normal values on 100 mg. of vitamin. The solid line represents the capillary resistance values, the broken line the concentration of vitamin in the blood plasma, the hollow columns the intake, and the solid columns the amount excreted in twenty-four hours. The patient suffered from hyperemesis gravidarum, and had epistaxis and hemorrhagic gums. According to the history her diet had been adequate until six weeks before treatment; the deficient diet was extremely restricted.

chloracetic acid (C.P.). These proportions require less dilution of the plasma than others,[6] and we believe contribute toward a more accurate determination.* The filtrate was titrated against 2:6 dichlorophenolindophenol. The dye was expressed from a screw pipette graduated to 0.002 c.c. Two-tenths cubic centimeter of the filtrate was used and the titrations carried out in triplicate in conjunction with a triplicate blank. The blank was prepared by adding 0.9 c.c. 20 per cent trichloracetic acid to 2.1 c.c. distilled water. This latter precaution is necessary because of the small amount of vitamin being titrated. The subtraction of the blank value from the unknown

*It is realized that this method is not as satisfactory in infants as that of Farmer's.

TABLE I

RESULTS OF VARIOUS TESTS FOR VITAMIN C DEFICIENCY IN NORMAL INDIVIDUALS AND OTHERS HAVING HEMORRHAGIC DIATHESIS

(When two values appear in one column, the first is the initial value, the second the final value after treatment with cevitamic acid)

CASE	ANEMIA	FASTING URINE MG. PER CENT	FASTING BLOOD MG. PER CENT	CAPILLARY RESISTANCE CM. HG NEG. PRESSURE	SIX HOUR EXCRETION TOTAL MG.	4 HR. BLOOD ABSORPTION MG. PER CENT	REMARKS
Normal Case 1 No bleeding	None	1.67	0.87	40	46	2.10	
Normal Case 2 No bleeding	None	2.50	0.69	45	45	1.95	
Normal Case 3 No bleeding	None	0.97	0.62	55	46	2.05	
Normal Case 4 No bleeding	None	14.00 12.00	1.06	30	41	2.05	
Normal Case 5 No bleeding	None	1.90	0.68	55	41	2.0	
Case 6 Thrombocytopenic* purpura—Epistaxis	None	2.30	0.95	10 10	50	No test	Oral R; bleeding continued; no change in capillary resistance
Case 7 Familial epistaxis	None	2.37	1.60	45	66	No test	Bleeding continued; no prolonged R
Case 8 Mildly deficient, bleeding gums	None	0.30 4.28	0.33 1.08	40 45 plus	21 57	1.05 2.30	Oral R; bleeding ceased fourth day
Case 9 Mildly deficient, bleeding gums, and epistaxis	None	0.70 3.32	0.14 1.22	35 45 plus	27 53	1.05 2.15	Oral R; bleeding ceased sixth day
Case 10 Mildly deficient, epistaxis	None	1.90 1.94	0.30 1.12	30 45 plus	25 52	1.05 2.40	Oral R; bleeding ceased before R
Case 11 Hypertension with epistaxis	Slight	0.60 2.37	0.52 1.49	15 45 plus	25 55	1.30 2.20	I.V. R†; bleeding ceased third day; maintenance on oral O.J.‡ six months
Case 12 Hypertension with epistaxis	Marked	0.60 5.51	0.32 1.17	No response	17 55	No test	I.V. R; bleeding ceased fourth day; maintenance on oral O.J. five months
Case 13 Hypertension with epistaxis	None	1.13 0.86 0.90	0.55 0.56 0.25	25 45 20 No treatment	28 —	1.59 —	I.V. R; bleeding ceased third day; no maintenance R

*Urinary excretion based on a test dose of less than 15 mg. per kg.
†Intravenous treatment.
‡Orange juice.

‡Intravenous treatment.
§Orange juice.

TABLE I—CONT'D

Case 14 Gastric ulcer, massive hemorrhage	Marked	0.97	2.59	0.13	0.75	No response		13	41	0.69	1.45	I.V. R; stool positive until sixty-fifth day; failure of maintenance on oral O.J.
Case 15 Duodenal ulcer, massive hemorrhage	Marked	1.00	2.88	0.50	1.27	No response		4	70	No test		I.V. R; stool positive until ninth day; maintenance on oral O.J. five months
Case 16 Duodenal ulcer, weeping	Slight	0.85	1.10	0.35	0.55	35	45	7	33	1.49	1.25	Partial I.V. R; discharged without follow-up
RANDOM CASES												
Case 17 Hyperemesis gravidarum, epistaxis, bleeding gums	Slight	0.40	—	0.09	1.05	25	45 plus	No test		No test		Vomiting ceased third day; bleeding ceased fourth day; oral R and maintenance
Case 18 Scarlet fever, epistaxis, venipuncture petechiae	None	1.62	2.48	0.20	1.11	15	45 plus	10	53	0.55	1.76	Bleeding ceased first day; decrease in number of petechiae; oral maintenance six months; menstrual periods decreased from nine to five days
Case 19 Esophageal ulcer, menorrhagia	None	0.90	1.85	0.47	1.01	25	45 plus	23	No test	No test		Maintained five months
Case 20 Idiopathic cellulitis, ankle	None	0.42	2.47	0.42	1.15	25	45 plus	23	51	0.95	2.10	Increased rate of healing; maintenance of increased values on oral O.J. three months
Case 21 Varicose veins, ecchymotic hemorrhage	None	1.60	2.73	0.18	1.23	10	45	4.5*	50	No test	1.76	Increased rate of healing; maintenance of improvement on oral O.J. eight months
Case 22 Hodgkin's disease, epistaxis, melena	Marked	0.80	1.15	0.064	0.45	No response		8	32	No test		Stool variable, never neg. in spite of extensive oral and I.V. treatment
						No response		8*	32	No test		
Case 23 Severe scurvy, extensive bleeding into skin and bowel	Slight	0.40	4.40	0.064	1.23	5	45 plus	2*	40	No test		I.V. R; progressive healing maintenance on oral tomato juice
Cases 8-23 Averages for Scorbutic Group	High	1.90	5.51	0.55	1.49	40		28	70	1.59	2.40	
	Average	0.88	2.55	0.28	1.04	24	45 plus	16	49	0.98	1.86	
	Low	0.30	0.86	0.064	0.45	5		2	32	0.09	1.20	

makes an appreciable difference in the end result. This may explain the lower values in this study in contrast to those of other workers. The lower limit of normal for this method is 0.5 mg. per cent. The average normal fasting value ranges between 0.8 and 1.00 mg. per cent.

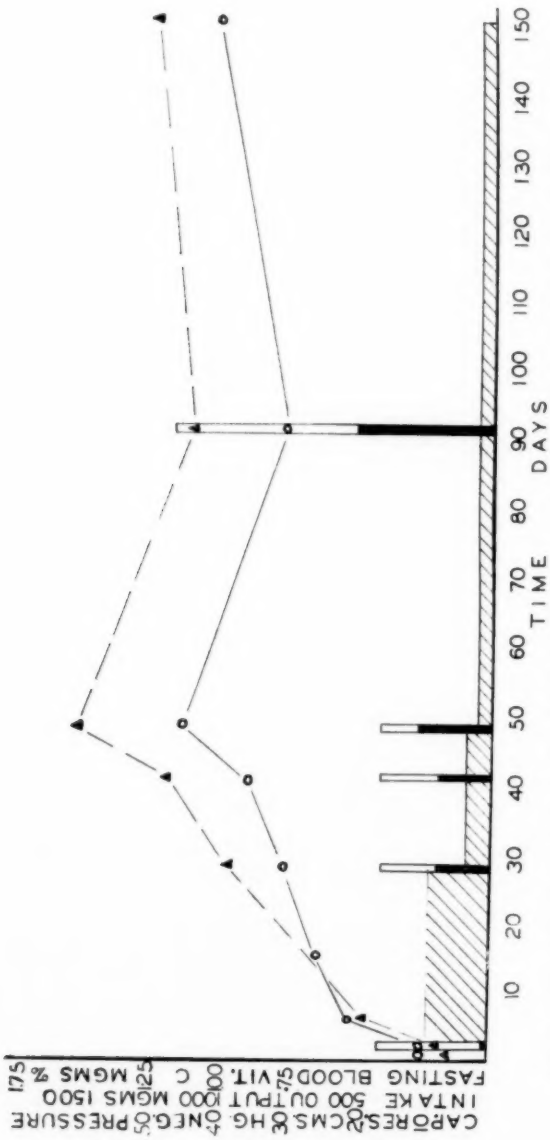
CAPORESS 2 CMS. OF HG. ONE G. OF PRESSURE
INTAKE 500 OUTPUT 1000 MGMS 1500
FASTING BLOOD VIT. C MGMS %
175
125
100
75
50
25
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
TIME DAYS

Fig. 4.—Progressive increase in fasting blood concentration of vitamin C (broken line), capillary resistance (solid line), and urinary excretion test following alternating oral and parenteral administration of the vitamin to a patient whose complaint was ecchymotic hemorrhages of her legs. Remarkable subjective improvement in this case coincidental with the changes in the values shown as well as increased rate of repair of an indolent ulcer. Symbols as in Fig. 3. The shaded columns represent oral intake of vitamin as orange juice.

Blood Absorption Curves.—Specimens of blood were taken from the other arm immediately following injection of vitamin and again at two, five, and ten minutes and other intervals up to six, eight, and twenty-four hours, to thoroughly establish the type of curve. Later the number of specimens was decreased to three or four. These were taken at five minutes, one, two, and

four hours. The last specimen is required to note changes in the blood curves of the mildest deficiencies, where a deviation from normal takes place only at this time.

ANALYSIS OF RESULTS

For the sake of brevity the results have been condensed in a single table in which the original values and those following treatment alone are shown.

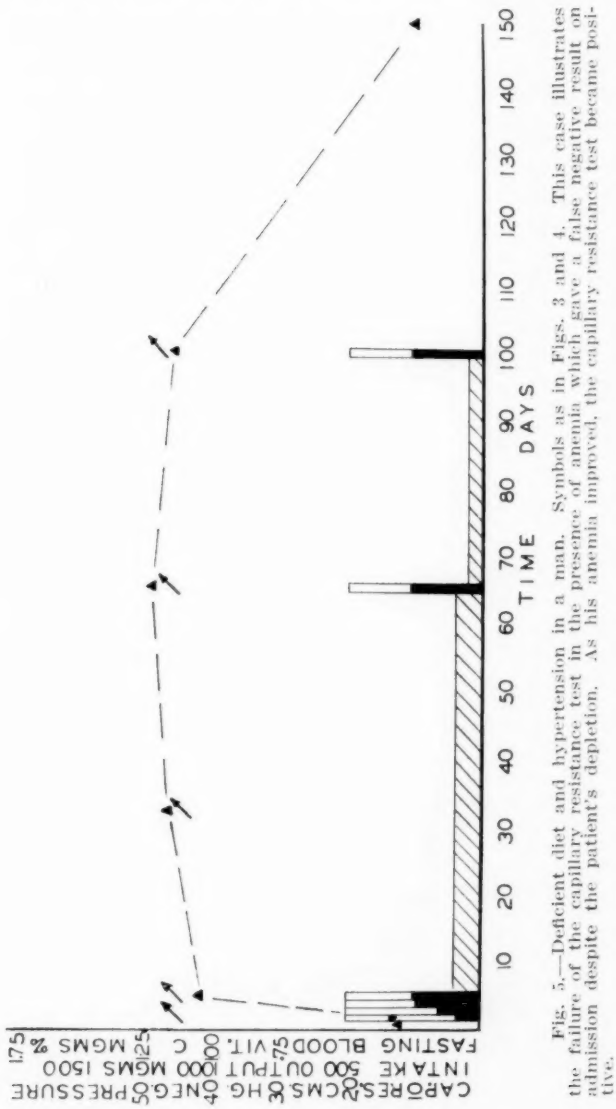
CAPORES. CMS. OF HG. ONE G. PRESSURE
INTAKE 500 OUTPUT 1000 MGMS 1500
FASTING BLOOD VIT. C MGMS %
175
25
00
75
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
TIME
DAYS

Fig. 5.—Deficient diet and hypertension in a man. Symbols as in Figs. 3 and 4. This case illustrates the failure of the capillary resistance test in the presence of anemia which gave a false negative result on admission despite the patient's depletion. As his anemia improved, the capillary resistance test became positive.

A number of cases are illustrated in more detail in four figures which are representative. From these it may be seen that the fasting urine values in the group of five normal adults was 1.95 mg. per cent, in the deficient group 0.88 mg. per cent, or somewhat less than half the amount. It may also be seen that there was no appreciable difference in vitamin C content of the

urine of slightly and moderately depleted patients, and that each group contained instances in which the value was greater than in the normal group from which we conclude that the examination of a single, fasting specimen of urine is not a safe criterion of subclinical scurvy or even mild scurvy, although the average value for a group will serve to reveal vitamin C deprivation.

Similarly, the fasting blood values clearly indicate vitamin C starvation in both deficient groups and in this limited series the maximum value recorded among the unsaturated patients was distinctly less than among the normals. This test also serves in a rough way to indicate the *degree* of deficiency, the average for the mildest cases being 0.36 mg. per cent and for the severer cases 0.20 mg. per cent. However, overlapping values were encountered in a number of cases in these two groups.

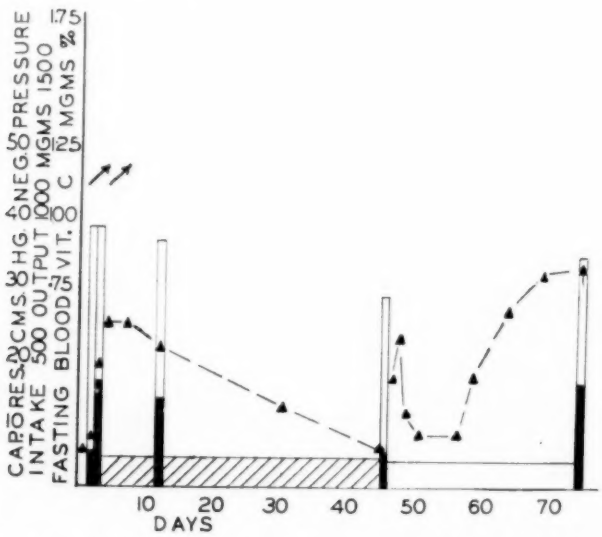

Fig. 6.—Failure to maintain saturation and normal blood values of vitamin C on an oral intake of 250 c.c. of orange juice daily. Recovery on the same amount given parenterally (following the forty-fifth day). The patients suffered from peptic ulcer and had had severe hemorrhage previous to treatment with vitamin C. Coincidental with treatment the hemorrhages ceased. Symbols as in Figs. 3 and 4. False negative capillary resistance due to anemia.

It is of considerable interest to observe how well the capillary resistance test measured the presence of vitamin C depletion and the degree of depletion. The average for the normals was 45 cm. Hg, for the mildest cases 29 cm., and for the severer cases 14 cm. In the one outspoken case of scurvy the lowest value of the entire group was secured, 5 cm. Hg negative pressure. However well the test performed in terms of group averages, a number of cases were studied in which it was not applicable and one in which it gave misleading results. Thus four patients had no petechiae at maximum pressure, although they were all severely depleted. The results of the saturation tests leave little doubt on this point. Whether these errors are due to anemia, present to a marked degree in each case, as we believe, or whether the scorbutic hemorrhagic diathesis was actually not present and the chemical tests at fault can-

not be considered as established from our experience. What observations have been made support the former view, because in one case capillary fragility was later demonstrated as the anemia improved. The false positive test occurred in the single case of thrombocytopenic purpura in the series. False results in such cases have previously been recognized as a limitation of the test.[3, 7]

The capillary test lends itself to therapeutic trial and in each instance in which it was positive, i.e., below 30 cm. Hg, it promptly increased under treatment just as it reversed its trend if the body's supply of vitamin was again depleted.

Extremely satisfactory results were secured by the measurements of saturation. The urinary tests show an average value of 44 per cent of the test dose excreted within six hours by normal persons, only 27 per cent by mildly deficient ones, and 16 per cent by the severer cases. Moreover, no overlapping occurred in any of the groups, although this point will not stand much emphasis because the classification of these cases into various groups is not completely satisfactory. Equally satisfactory results were secured by the blood saturation curves. The results with this procedure appear to be the most precise measure of saturation available.

It is apparent, however, that a critical attitude requires more study of cases similar to No. 14, 15, and 16, before accepting the results of blood saturation tests completely. These patients were characterized by severe hematemesis and several of the determinations made shortly after hemorrhage. Since an appreciable blood loss means likewise a loss of vitamin C and the lag period in which the blood replaces its vitamin C from body stores is unknown, it is evident that recovery in cases following massive hemorrhage should be a controlled factor in any calculations of the kind reported. However, such qualifications apply even more to the measurement of vitamin C in single blood samples. The entire problem of vitamin C balance in patients with peptic ulcer is worthy of further investigation.

In summary it would seem that of the single determinations the blood assay is the most informative but that the saturation tests yield more precise, quantitative information. The added difficulties of the latter preclude their use in routine work, while the simple blood examination is one easily adaptable to hospital routine. For thorough study of the vitamin C reserves and rate of consumption, the saturation tests, and particularly the blood curve, are more satisfactory.

It is probable that these procedures will supplant the capillary resistance test because of their greater dependability. However, some expertness and experience with this simple clinical method make it a simple matter to recognize the vast majority of cases of vitamin C starvation, and if the readings are made during a course of treatment and an appreciable recovery in capillary resistance occurs, definite information is readily acquired at the bedside as to the vitamin C status of the subject.

In the present series the capillary resistance test has, for example, been of great usefulness in demonstrating that such a group of cases as those

classified as mildly scorbutic had not only chemical evidence of vitamin C depletion but also objective evidence of morbid effect of the depletion. While the series is small it seems to represent substantial evidence that these mild cases of scurvy are in a measurable way suffering pathological effects of their deficiency. If those effects are of any great moment or not, we cannot say.

CONCLUSIONS

1. All of the procedures we have used will, subject to their individual limitations, detect subclinical scurvy.

2. The chemical tests seem somewhat more sensitive of very mild deficiencies than the capillary test.

3. The correlation between tests of saturation and of morbid function as expressed by lowered capillary resistance are nevertheless close enough that we may assume from the former that a morbid condition does in fact exist.

4. The capillary resistance test is the simplest procedure. It may be tested at the bedside and, in the majority of cases, gives dependable information concerning the presence or absence of vitamin C depletion. Its greatest drawback in adults in the present study was the occurrence of falsely negative results due to severe anemia.

5. The assay of the vitamin content of the blood is the simplest dependable procedure. It roughly indicates the *degree* of depletion.

6. The assay of a single sample of urine is a rough and frequently false measure of vitamin saturation.

7. All of the methods yield superior results when used in conjunction with a therapeutic test.

8. The most precise and dependable method of determining the degree of saturation of vitamin C is the determination of the rate of absorption of an injected dose from the blood stream. The rate of excretion of a test dose is less precise but quite dependable.

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THE STAINING REACTIONS OF FATS AFTER THE USE OF VARIOUS DYES AND FIXING AGENTS*

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THERE has been considerable interest in the past twenty years in the tissue responses induced by various fats after reaching the lungs intratracheally, either by accidental aspiration, or through various experimental, diagnostic, and therapeutic measures. In other tissues also, pathologists have studied "paraffinomas" and other types of reaction to foreign lipids in contact with living body cells. It has become increasingly important to be able to recognize the nature of the foreign lipid and to identify it, if possible, by histologic methods. In the study reported in this paper, the staining reactions of a number of common food and medicinal fats, in fresh and fixed states, when treated by the common fat dyes, have been investigated. The results are presented in tabular form to assist, so far as is possible, in the identification of lipids under such circumstances.

In 1917, Waters, Bayne-Jones and Rowntree[1] injected a mixture of iodoform and olive oil into the tracheas of animals. They found a proliferative type of bronchopneumonia in the lungs of animals thus treated, and demonstrated the presence of fat in the alveoli of the lungs by fixing the tissue in 40 per cent formaldehyde solution and staining with scharlach R. In 1920, Guieysse-Pellissier[2] also injected olive oil intratracheally into the lungs of rabbits and dogs and described the cellular tissue response. Corper and Freed,[3] in 1922, published the results of intratracheal injections of chaulmoogra oil, olive oil, and liquid petrolatum. They observed that olive oil and liquid petrolatum were retained for months in the alveoli of the lungs where they are capable of producing a proliferative bronchopneumonia.

In 1926, Kaufmann and Lehmann[4] published an extensive study of fatty acids, neutral fats, and lipoids in an attempt to determine the possibility of specific differentiation of fatty substances by their morphological staining reaction to various fat dyes. They stained saturated and unsaturated fatty acids, several neutral fats, and various lipoid substances alone and in various combinations, using four fat stains: sudan III, Nile blue sulfate, Fischler's method (copper acetate and hematoxylin), and the Smith-Dietrich method (potassium bichromate and hematoxylin). They found that both butter and lard stain a golden yellow with sudan III, a rose color with Nile blue sulfate, and were unstained with the Smith-Dietrich method. They concluded that fat stains are not specific as a means of identification even for groups of fatty substances, to say nothing of the specific identification of a single fat. They

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did observe, however, that the blue color with Nile blue sulfate is accentuated according to the degree of unsaturation of the fatty acids and that this dye is a specific means of differentiating neutral fats from fatty acids, the former staining a rose red, even though the rose red is eventually dominated by blue, and the latter a blue.

Pinkerton,[5] in 1927, presented a comprehensive study of pneumonia produced by the aspiration of oils and fats, together with observations made at autopsy upon six clinical cases. In the following year[6] he reported experimental data on the reaction of oils and fats in the lung as investigated by the intratracheal injection of puppies and rabbits with iodized vegetable oils, olive oil, chaulmoogra oil, cod liver oil, milk fat, lard oil and mineral oil. He recorded the solubility, refraction and staining reactions as a means of identification of the various fatty substances. He found that lipiodol, having a poppy seed oil base and containing 40 per cent iodine, stained red with scharlach R and showed no appreciable tissue reaction in the lung, thus being most suitable for roentgenologic purposes. Olive oil stained brown with osmic acid, red with scharlach R, and light pink with Nile blue sulfate. Butter stained brown with osmic acid, orange red with scharlach R, and pink with Nile blue sulfate. Lard oil was found to produce rapid necrosis of the lung tissue. Mineral oil was found not to stain with osmic acid, but did stain orange red with scharlach R, pink with Nile blue sulfate, and was not dissolved by absolute alcohol in formaldehyde-fixed frozen sections.

Ikeda,[7] in 1935, published an extensive review of the experimental and clinical work done in this field; he reported seven more clinical cases of oil aspiration pneumonia collected from a series of 101 necropsies performed on children, and also the results of experimental work done by himself on the intratracheal injections of oils and fats into animals. He stated that liquid petrolatum can be identified through its pale orange yellow staining with scarlet red and its negative reaction to osmic acid and Ciaccio's stain. He found that mineral oil and its various combinations (including certain medicated oils used for nasal instillations), cod liver oil, halibut liver oil, milk and cream, castor oil, and iodized poppy seed oil are the chief causal factors in the production of oil aspiration pneumonia.

METHOD

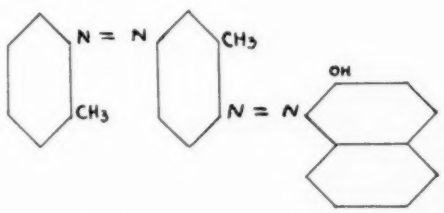
In our present investigation we were interested particularly in determining the influence of various fixing agents on the staining reactions of fatty substances commonly aspirated into the trachea and lungs. Distribution of the fat in lung tissue was obtained by the intravenous injection of 1 c.c. of the liquid fatty substance in a white rat. The fat emboli thus produced in the lungs caused an immediate asphyxial death. Representative pieces of the lung were then placed in the following solutions: normal saline for the unfixed fresh frozen sections, 10 per cent formol solution, saturated aqueous solution of mercuric chloride, and Müller's fluid. The tissues in 10 per cent formol solution were allowed to fix for twelve hours, were then rinsed in water and sectioned on the freezing microtome. After twelve hours in the

mercuric chloride fixing solution, tissues were washed for twenty-four hours before freezing and sectioning. For Müller's fixation the tissues were allowed to lie in the fixing solution for three weeks, the solution being changed whenever it became cloudy. They were then washed for twenty-four hours in water prior to freezing and sectioning. The preparation of the fixing solutions and staining solutions and the technique of staining were as given by Warthin,[8] with the exception that no counterstain was used with osmic acid and indophenol.

MATERIALS

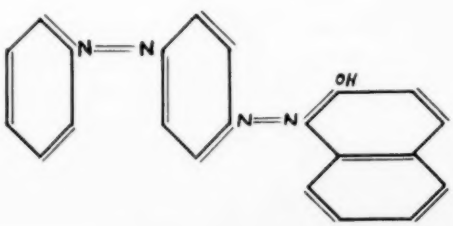
The formol solution employed in this experimental work is slightly acid in character and identical with that used in our routine diagnostic work. Our findings agree with those of Millot and Giberton[9] who found that formol has an hydrolyzing effect on fatty tissues. In our sections fats, especially the unsaturated vegetable oils, after being fixed in formol, stained a brilliant deep magenta with Nile blue sulfate. However, they began to turn blue in the course of a few days, indicating the appearance of fatty acids. Also pieces of lung containing the unsaturated vegetable oils, when left in formol for several weeks and then sectioned and stained, gave an immediate deep blue color with Nile blue sulfate. A short account of the fat dyes used is given below.

Scharlach R (scarlet red, sudan IV) is a weakly acid oil soluble dye of the azo group, a dimethyl derivative of sudan III, with the formula according to Kinosita[10] as given below:



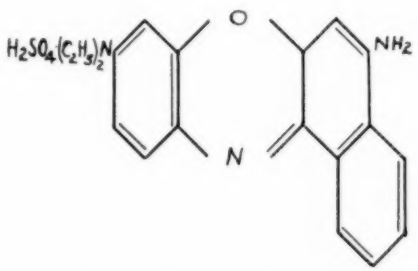
This dye in a saturated solution of equal parts of 70 per cent alcohol and acetone stains fat various tints and shades of scarlet red. The color is permanent. In comparison with sudan III, it appears that scharlach R stains fat a scarlet instead of an orange red, because of the substitution of methyl radicals as indicated in the structural formula.

Sudan III (sudan G, scarlet G) is a weakly acid, fat soluble dye of the azo group, with a structural formula as given below, according to Kaufmann and Lehmann:[4]



This dye in a saturated solution of equal parts of 70 per cent alcohol and acetone stains fats various tints and shades of orange. This stain is permanent. There is no diffusion of the dye into the glycerin-gelatin mounting medium, with either sudan III or scharlach R.

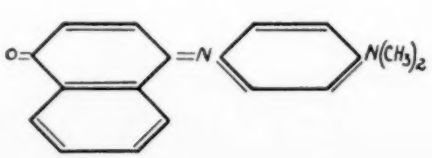
Nile blue sulfate (Lorrain Smith fat stain) is a basic dye of the oxazin series, with the following structural formula, according to Kaufmann and Lehmann:[4]



In its preparation for the staining of fats the dye is boiled with dilute sulfuric acid and is thus partially converted into a new dye of the class known as oxazones. The oxazone portion of the dye is red and readily fat soluble. Nile blue sulfate itself combines readily with fatty acids, staining them blue. As a result Nile blue sulfate is used to distinguish between free fatty acids and neutral fats, staining the former a blue and the latter a red. The formol-fixed preparations are permanent only for a few days to a few weeks, while sections from unfixed tissues and from those fixed in mercuric chloride and Müller's fluid are permanent for several months. There is a moderate amount of diffusion of the dye into glycerin-gelatin mounting media.

Osmic acid (perosmic acid, osmic anhydride, osmium tetroxide, OsO_4) is reduced to the lower oxide or to black, metallic osmium, especially by the unsaturated fats and fatty acids. McClung[11] states that the saturated fats and fatty acids do not reduce osmic acid. When sections containing unsaturated fatty substances are placed in a 1 per cent aqueous solution of osmic acid for one hour, they are permanently browned or blackened. There is slight diffusion of the reduced osmium into the mounting media, giving it a brownish discoloration, more marked when the fat is very abundant and especially if the washing has not been thorough. A special application of osmium tetroxide depends upon the fact that as it is reduced to a lower oxide by some of the true fats and some lipoids, it renders these substances less soluble in alcohol, xylol, benzol, clearing oils, and balsam. This makes it possible, by using a rapid paraffin method, to secure paraffin sections having the "osmicized" fat still intact.

Herxheimer,[12] in 1901, published his results from several fat-staining methods, including indophenol. He gives the following structural formula for indophenol:



He obtained a distinct blue color by staining fat twenty minutes in a saturated solution of indophenol in 70 per cent alcohol. No counterstain was used. The preparations of this dye mounted in glycerin-gelatin are permanent for several months and the dye shows no diffusion into the mounting media.

A short account of each oil or fat used in the present investigation precedes a tabulation of the staining results obtained with it. Iodine numbers are as given by Hodgman.[13] These are of value since they serve to indicate the degree of saturation of the fats, which is an important factor in influencing the staining quality.

TABLE I

POPPY SEED OIL

FAT DYE	FRESH, UNFIXED	FORMOL	MERCURIC CHLORIDE	MÜLLER'S FLUID
Scharlach R	Deep orange red to deep brilliant scarlet	Deep orange red to very deep brilliant scarlet	Medium brilliant orange red	Deep brilliant orange red to deep scarlet
Sudan III	Bright orange to brilliant medium red orange	Brilliant deep orange to deep brilliant red orange	Medium brilliant red orange	Bright orange to deep brilliant red orange
Nile Blue Sulfate	Light to deep brilliant magenta	Medium to very deep brilliant magenta	Light brilliant magenta	Medium to deep brilliant magenta
Osmic Acid	Black	Black	Black	Black
Indophenol	Pale to deep blue	Deep blue to blue black	Very deep blue	Deep blue to blue black

TABLE II

LIPIODOL (20 PER CENT IODINE)

FAT DYE	FRESH, UNFIXED	FORMOL	MERCURIC CHLORIDE	MÜLLER'S FLUID
Scharlach R	Deep orange red to deep brilliant scarlet	Deep orange red to very deep brilliant scarlet	Medium brilliant orange red	Deep brilliant orange red to deep scarlet
Sudan III	Bright orange to brilliant medium red orange	Brilliant deep orange to deep brilliant red orange	Medium brilliant red orange	Bright orange to deep brilliant red orange
Nile Blue Sulfate	Light to deep brilliant magenta	Medium to very deep brilliant magenta	Light brilliant magenta	Medium to deep brilliant magenta
Osmic Acid	Black	Black	Black	Black
Indophenol	Pale to deep blue	Deep blue to blue black	Very deep blue	Deep blue to blue black

Poppy seed oil (from *Papaver somniferum*) is a bland fixed oil with an iodine number of 128 to 141. The uses of poppy seed oil are similar to olive oil, but in addition it is used as the oil base of lipiodol for roentgenologic purposes. No difference was observed between the staining reactions of poppy seed oil and *lipiodol* containing 20 per cent iodine.

Olive oil, from *Olea europaea* and commonly known as sweet oil, is a bland fixed oil with an iodine number of 79 to 88. It is used as a laxative and as an emollient in corrosive poisoning, thus lending itself to possibilities of aspiration into the lungs.

TABLE III

OLIVE OIL

FAT DYE	FRESH, UNFIXED	FORMOL	MERCURIC CHLORIDE	MÜLLER'S FLUID
Scharlach R	Deep orange red to medium brilliant scarlet	Deep orange red to deep brilliant scarlet	Light brilliant orange red	Medium brilliant orange red to brilliant scarlet
Sudan III	Bright orange to medium brilliant red orange	Bright orange to deep brilliant red orange	Light to medium brilliant red orange	Medium to deep red orange
Nile Blue Sulfate	Light to deep brilliant magenta	Medium to deep brilliant magenta	Light brilliant magenta	Medium to deep blue magenta
Osmic Acid	Black	Black	Black	Black
Indophenol	Pale to deep blue	Deep blue to blue black	Purple to deep blue	Deep blue to blue black

Lard (adeps suillus) has an iodine number of 62.5 to 79. Lard is chiefly aspirated while being taken as food.

TABLE IV

LARD

FAT DYE	FRESH, UNFIXED	FORMOL	MERCURIC CHLORIDE	MÜLLER'S FLUID
Scharlach R	Deep brilliant orange red	Very deep brilliant orange red	Light to medium brilliant orange red	Very deep briliant orange red
Sudan III	Light brilliant red orange	Medium brilliant red orange	Very light to medium red orange	Medium brilliant red orange
Nile Blue Sulfate	Light to medium brilliant magenta	Medium brilliant magenta	Very light brilliant magenta	Medium brilliant magenta
Osmic Acid	Dark brown to black	Dark brown to black	Dark brown to black	Dark brown to black
Indophenol	Very pale blue to blue black	Dark blue to black	Deep blue to blue black	Dark blue to blue black

Butter fat (Vaccae lactis adeps) is a complex animal fat having an iodine number of 26 to 28. Milk fat is commonly aspirated by infants in feeding. It may reach the lungs accidentally in the tube feeding of psychopathic patients, or may be aspirated as vomitus.

Liquid paraffin (mineral oil, petrolatum liquidum) and white paraffin (solid paraffin) belong to the series of saturated hydrocarbons. They are of particular interest here because of their relation to lipoid pneumonia and to "paraffinomas." Mineral oil may reach the bronchial tree while serving as a vehicle for medication of the upper respiratory tract.

TABLE V

BUTTER FAT

FAT DYE	FRESH, UNFIXED	FORMOL	MERCURIC CHLORIDE	MÜLLER'S FLUID
Scharlach R	Light to medium deep brilliant orange red	Deep brilliant orange red	Medium brilliant orange red	Deep brilliant orange red
Sudan III	Light to medium brilliant red orange	Medium brilliant red orange	Light brilliant red orange	Light to medium brilliant red orange
Nile Blue Sulfate	Pale to medium brilliant magenta	Light to medium brilliant magenta	Very light brilliant magenta	Light to medium brilliant magenta
Osmic Acid	Dark gray to black	Dark gray to black	Dark gray to black	Dark gray to black
Indophenol	Light blue to blue black	Dark blue to blue black	Deep blue to blue black	Dark blue to blue black

TABLE VI

MINERAL OIL

FAT DYE	FRESH, UNFIXED	FORMOL	MERCURIC CHLORIDE	MÜLLER'S FLUID
Scharlach R	Pale orange to medium brilliant red orange	Medium orange to brilliant deep red orange	Light brilliant orange	Light to medium brilliant red orange
Sudan III	Very pale orange yellow	Pale orange yellow	Light pale orange	Pale orange yellow
Nile Blue Sulfate	Pale light pink	Bright light pink	Very pale pink	Light pale pink
Osmic Acid	Unstained	Unstained	Unstained	Unstained
Indophenol	Very pale blue	Pale blue	Pale purple	Pale purple

TABLE VII

PARAFFIN (SOLID, MELTING POINT 40° TO 42° C.)

FAT DYE	FRESH, UNFIXED	FORMOL	MERCURIC CHLORIDE	MÜLLER'S FLUID
Scharlach R	Unstained	Unstained	Unstained	Unstained
Sudan III	Unstained	Unstained	Unstained	Unstained
Nile Blue Sulfate	Very light pale pink	Very light pale pink	Very light pale pink	Very light pale pink
Osmic Acid	Light to dark gray	Light to dark gray	Light to dark gray	Light to dark gray
Indophenol	Very pale blue	Very pale blue	Very pale blue	Very pale blue

TABLE VIII

COMPARISON OF THE STAINING REACTIONS AFTER FIXATION WITH FORMOL AND WITH MERCURIC CHLORIDE

FAT DYE AND FIXATION	POPPY SEED OIL AND LIPIODOL (20%)	OLIVE OIL	LARD	BUTTER FAT	MINERAL OIL	PARAFFIN
Scharlach R Formol	Deep orange red to very deep brilliant scarlet	Deep orange red to deep brilliant scarlet	Very deep brilliant orange red	Deep brilliant orange red	Medium orange to brilliant deep red orange	Unstained
Mercuric Chloride	Medium brilliant orange red	Light brilliant orange red	Light to medium brilliant orange red	Medium brilliant orange red	Light brilliant orange	Unstained
Sudan III Formol	Brilliant deep orange to deep brilliant red orange	Bright orange to deep brilliant red orange	Medium brilliant red orange	Medium brilliant red orange	Pale orange yellow	Unstained
Mercuric Chloride	Medium brilliant red orange	Light to medium brilliant red orange	Very light to medium red orange	Light brilliant red orange	Light pale orange	Unstained
Nile Blue Sulfate Formol	Medium to very deep brilliant magenta	Medium to deep brilliant magenta	Medium brilliant magenta	Light to medium brilliant magenta	Bright light pink	Very light pale pink
Mercuric Chloride	Light brilliant magenta	Light brilliant magenta	Very light brilliant magenta	Very light brilliant magenta	Very pale pink	Very light pale pink
Osmic Acid Formol	Black	Black	Dark brown to black	Dark gray to black	Unstained	Light to dark gray
Mercuric Chloride	Black	Black	Dark brown to black	Dark gray to black	Unstained	Light to dark gray
Indophenol Formol	Deep blue to blue black	Deep blue to blue black	Dark blue to black	Dark blue to blue black	Pale blue	Very pale blue
Mercuric Chloride	Very deep blue	Purple to deep blue	Deep blue to blue black	Deep blue to blue black	Pale purple	Very pale blue

It has been known for a long time that there is a close relationship between the degree of unsaturation of lipids and the character and intensity with which they are stained by the fat dyes. Mulon,[14] in 1904, investigated the staining action of osmic acid on fats. He found that fats which were immediately blackened by osmic acid were, for the major part, unsaturated triolein; while those that were made only yellow or brown were largely saturated palmitin and stearin. Butter, which contains 60 to 70 per cent palmitin and 30 to 40 per cent olein, is somewhat more saturated than is lard because of the presence of a greater number of unsaturated esters of oleic and linoleic acid in the latter fat. This difference is made manifest by lard staining a slightly deeper color than does butter. Triolein, which is the chief constituent of olive oil, contains three molecules of the unsaturated oleic acid, and poppy seed oil is even more unsaturated than is olive oil. The latter two vegetable oils were blackened very quickly and deeply with osmic acid and stained very intensely with all of the other fat dyes used.

CONCLUSIONS

1. Fresh frozen unfixed tissues are highly satisfactory for the staining of neutral fats. The unfixed fat, in general, stains a slightly lighter color than that fixed in formol.

2. Formol-fixed frozen sections are the most satisfactory for the routine demonstration of fat for the following reasons: The tissue does not require extensive washing before cutting; the formol hardens the fat; the sections are easily handled, and they stain more deeply with scharlach R, sudan III and Nile blue sulfate. Also formol-fixed tissues can be used for myelin staining. Unfortunately, probably because of the development of formic acid, formol-fixed tissues, both in the mass and in sections, may suffer partial hydrolysis of some of the lipids. This liberates free fatty acids and makes it impossible to demonstrate the presence of neutral fats with Nile blue sulfate in tissue sections made from gross specimens fixed in formol, and also causes the red color in formol-fixed preparations, previously stained with Nile blue sulfate, to be gradually dominated by blue. This change may occur within a few days with the unsaturated vegetable oils.

3. After fixation in mercuric chloride, as compared to formol fixation, fats stain considerably lighter with scharlach R, sudan III, Nile blue sulfate, and a more distinct deeper blue with indophenol. The preparations of Nile blue sulfate and indophenol that have been fixed in mercuric chloride are more permanent than those fixed in either Müller's fluid or formol. The necessity for thorough washing and the danger of overfixation are disadvantages of this method.

4. After fixation in Müller's fluid the neutral fats give beautiful results, staining very well with all the fat dyes used. The color is lighter than with formol and deeper than with mercuric chloride. No essential change is noted with osmic acid as related to this fixative. Müller's fixing fluid has the disadvantage of requiring prolonged fixation and extensive washing of the tissue. It is a good preservative of fat, but not quite so good as mercuric chloride, as shown by Nile blue sulfate.

5. The neutral fats studied, in general, stain according to their degree of unsaturation. Poppy seed oil, the most unsaturated of the oils studied, stains the deepest, and is then followed by olive oil, lard, butter fat, and the saturated hydrocarbons, including mineral oil and solid paraffin, staining progressively lighter in the order named.

6. There are multiple factors involved in the staining of fat, which make it impossible to name a specific shade or tint as uniformly characteristic of a particular fat.

7. Paraffin (solid) can be identified with a moderate degree of certainty by its negative reaction to scharlach R and sudan III, its pink crystalline character with Nile blue sulfate, and its gray color with osmic acid.

8. Mineral oil (petrolatum liquidum) can be identified by its pale to deep red orange staining with scharlach R and its negative reaction to osmic acid.

9. Within the limited group studied, the vegetable oils stained somewhat more deeply with all of the dyes used than did the animal fats.

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THE EFFECTS OF LIGATION OF THE COMMON BILE DUCT ON THE BLOOD IODINE AND BLOOD COUNTS OF MALE RABBITS*

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BLOOD iodine has received growing attention in the last fifteen years as methods for its determination have been improved. Interest has centered chiefly on the relation of the thyroid, particularly the pathologic gland, to blood iodine; but it has become increasingly evident that factors other than the thyroid play important roles in regulating blood iodine. Within the last five years Yuzuriha[17, 18] has experimentally demonstrated the considerable influence of the reticulo-endothelial system on iodine metabolism; Elmer[8] has reported on the role of the liver in the regulation of blood iodine; and Perkin[14, 15] has more recently commented on the effects of liver and biliary tract disorders on blood iodine. At the DeCourcy Clinic this aspect of iodine metabolism has been further investigated, and we have reported[5-7, 16] elevated blood iodine in some liver and gall bladder patients.

Some of these patients had been given substances containing large quantities of iodine for x-ray purposes, previous to withdrawal of blood for iodine analyses. We have nine reports on the blood iodine at definite periods after ingestion of 2.2 gm. of iodine contained in 9.5 gm. of "Cholepulvis," a preparation of tetraiodophenolphthalein with other materials. These provide evidence of the changes in blood iodine after administration of this product, although the data are not satisfactory because the administration of the dye and the dietary regime before withdrawal of blood for iodine analyses were not under our control, and because the exact pathology of these patients in some instances remains undetermined. These reports are given in Table I.

Reasons for elevated blood iodine values such as those in Table I have been suggested and discussed by DeCourcy[5-7] in regard to these patients and to other liver and gall bladder patients without records of abnormal iodine intake previous to withdrawal of blood for iodine analyses. The present investigation was designed to throw more light on the reasons for the elevated blood iodine found in patients of the latter class, i.e., those without records of abnormal iodine intake. The method of investigation that suggested itself was to produce pathologic conditions of the gall bladder, liver, and reticulo-endothelial system by ligation of the common bile duct in experimental

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TABLE I

BLOOD IODINE AFTER INGESTION OF 9.5 GM. OF "CHOLEPULVIS"

PATIENT NUMBER	BLOOD IODINE (GAMMA/100 ML.)	DAYS BETWEEN TAKING DYE AND TAKING BLOOD	DIAGNOSES
49	13.5	1	Acute hydrops of gall bladder
71	2100	1	Chronic cholecystitis with stones
129	3300	1	Chronic cholecystitis (?); chronic appendicitis
147	4.7	1	Chronic cholecystitis with stones
211	1300	1	Carcinoma of liver; carcinoma of ovary
138	49.5	10	Cholangeitis; infected biliary tract
89	110	13	Chronic cholecystitis with stones
90	87	14	Same as Patient 89, next day after intravenous glucose
135	180	14	Chronic cholecystitis with stones

animals and to follow their blood iodine while they lived on a diet containing only a small amount of iodine.

EXPERIMENTAL PROCEDURE

Thirty adult New Zealand albino male rabbits were obtained from a local breeder, housed in individual galvanized wire pens, placed on a diet (of known iodine content) of chopped alfalfa hay, oats and iodine-free water (water redistilled from potassium carbonate) with occasional cabbage, and weighed weekly.

Blood was drawn (with iodine-free syringes and needles) for iodine analyses at least fifteen hours after feeding time by heart puncture from all animals, except those with ligated bile ducts, from which blood was taken from marginal ear veins because the prolonged clotting time of their blood might have made heart puncture fatal. Mixed with dry, purified potassium oxalate, the blood was kept in a refrigerator until analyzed. In determining its iodine content by the procedure previously reported,[16] 5 ml. portions were generally used, except that samples drawn from ear veins were usually analyzed in 2 ml. portions because of the small quantity of blood obtained. Final samples from animals with ligated bile ducts were taken from their excised hearts and weighed instead of pipetted for analyses. Blood was customarily taken from the rabbits four to six hours before operation, fifteen hours after operation, and on the second, fourth, seventh, eleventh, fifteenth and twenty-first days after operation. Twice as much blood (11 ml.) was drawn from controls half as often up to fifteen days starting the day following operation of the other two rabbits in the group. Fig. 1 shows this. Erythrocyte, leucocyte, and differential counts on blood from marginal ear veins were taken each time after bleeding the animals.

The rabbits were divided into groups of three. The common bile duct of one of the three was ligated; the second animal was opened, its common bile duct located, and the animal sewn up without ligation of the duct; the third animal served as a nonoperated control existing on the same diet as the other two.

Operations were performed in the evening under aseptic conditions. The rabbits' ventral skin was sterilized with 70 per cent alcohol (diluted with iodine-free water from ethyl alcohol distilled from potassium carbonate), except that rabbits 1 and 2 were painted instead with tincture of merthiolate. Ether (analyzed for iodine) was used as anesthetic. The common bile duct was ligated at two places with light black silk (rabbits 1, 4, 10) or heavy

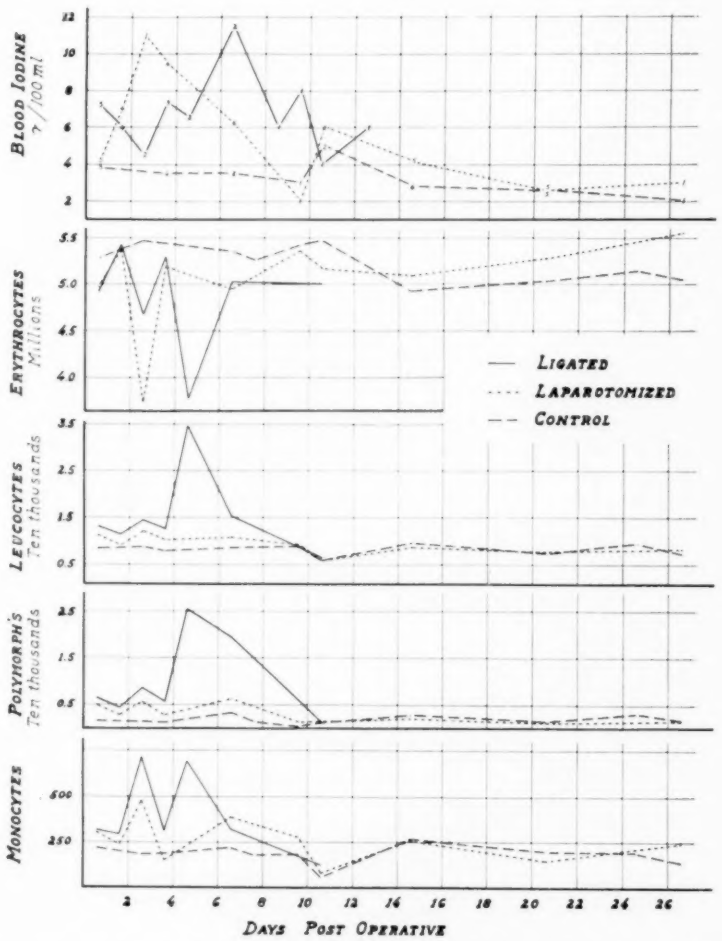

Fig. 1.—Average blood iodine content and average blood counts of rabbits after operation. The figures in the blood iodine curves indicate the number of animals observed at the different points.

white silk (rabbits 7, 13, 14, 17) and divided between the ligations, except in rabbit 17. Animals were sewn up with light black silk. Operations were found to have been accomplished as stated when the animals were examined after death.

RESULTS

a. *Blood Iodine.*—The results of the blood iodine analyses are recorded in Table II. Most of the figures given there are the averages of two analyses.

TABLE II

BLOOD IODINE OF RABBITS IN GAMMA PER 100 ML.

	RABBIT NO.																												
DATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
7/26		5																			4			21						
7/28	6		4																			2	3		15	15				
7/31				5																							21	28		
8/2					5																								14	20
8/4		2																			2									
8/6						5						6								9										
8/9	2																					3	3							
8/10	6†	9*	3																											
8/11	7†	11*						4	3								6													
8/12	2†			3	12							2																		
8/13		13*	2	20†	2*	6	2				2		3																	
8/14				8†	11*					3				8				4												
8/16		7*		8†	11*	4																								
8/17				3†																										
8/19		2*	3		2*		4	6		2	4		4																	
8/20							2†	4*	2	3†	3*	4																		
8/21							6†	2*		3†	4*																			
8/23					4*	7	6†	8*	4	8†	6*	4												12						
8/24		2*	2																											
8/26							8†	13*		15†	7*																			
8/27					9*	4																								
8/28										8†																				
8/30		2*	2				4†	8*	3					5						3										
9/1							6†																							
9/2					2*	4																								
9/3								5*	3						4	3			6											
9/8					3*	2																								
9/9								4*	2																					
9/13			2										4	4	3		1													
9/14		4	3*										5†																	
9/16														7†	11*															
9/18														10†																
9/20		3	3*												5*	4														
9/22														6†																
9/23																				3				6						
9/28		2	2*		4*	5									3*	2														
10/4		3	2*			3									3*	2	2	4												
10/29																	7†		7											

*Blood iodine of laparotomized rabbits.

†Blood iodine of rabbits with ligated bile ducts.

Certain of these figures have again been averaged and plotted in Fig. 1 to illustrate the variations of blood iodine after operation as compared with control figures. It will be noted that the blood iodine of the animals with ligated and divided ducts rose on the first day after operation and fell again, while that of the laparotomized animals rose on the second day and fell as the blood iodine of the animals with ligated and divided ducts again rose. Insufficient analyses were carried out to prove these differences in times of increase and decrease to be of statistical significance. A statistical analysis of the blood iodine findings is given in Tables III and IV.

TABLE III

GROUP OF RABBITS	DAYS AFTER OPERATION	NO. OF ANALYSES	MEAN VALUE (WITH PROBABLE ERROR) OF BLOOD IODINE IN GAMMA/100 ML.	STANDARD DEVIATION
A Preoperative controls		85	4.44 ± 0.16	± 2.24
B Postoperative controls	1-27	51	3.77 ± 0.18	± 1.89
C Animals with ligated and divided ducts	1-10	29	7.53 ± 0.51	± 4.10
D Laparotomized animals	1-10	30	7.63 ± 0.56	± 4.54
E Laparotomized animals	11-27	26	3.88 ± 0.37	± 2.83

TABLE IV

COMPARISONS OF MEAN VALUES FROM TABLE III

GROUPS OF RABBITS COMPARED	DIFFERENCE OF MEANS PROBABLE ERROR OF DIFFERENCE
A and B (pre- and postop. controls)	2.76
B and C (postop. controls and ligated animals, 1-10 days)	+ 6.92
B and D (postop. controls and laparotomized animals, 1-10 days)	+ 6.57
B and E (postop. controls and laparotomized animals, 11-27 days)	+ 0.27
C and D (ligated animals and laparotomized animals, 1-10 days)	0.13
D and E (laparotomized animals, 1-10 days and 11-27 days)	4.09

It will be noted from comparisons in Table IV that the blood iodine values of pre- and postoperative controls were not significantly different. The blood iodine of both the laparotomized animals and those with ligated and divided ducts showed a statistically significant difference from the controls but not from each other over the ten-day period following operation. After these ten days the laparotomized animals again had a normal blood iodine level, while most of the animals with ligated and divided ducts were dead.

TABLE V

GROUP OF RABBITS	DAYS AFTER OPERATION	NO. OF COUNTS	MEAN VALUE WITH PROBABLE ERROR AND STANDARD DEVIATION OF ERYTHROCYTE COUNT*	MEAN VALUE WITH PROBABLE ERROR AND STANDARD DEVIATION OF LEUCOCYTE COUNT
A Preop. controls		43	5606±34 ± 329	9866±130 ± 1266
B Postop. controls	1-27	27	5206±47 ± 358	8435±226 ± 1744
C Ligated animals	1-10	17	5074±89 ± 541	14353±986 ± 6171
D Laparotomized animals	1-10	21	5055±92 ± 625	10548±370 ± 2517
E Laparotomized animals	11-27	13	5204±84 ± 448	8442±323 ± 1727

*Last three figures omitted.

Blood iodine of laparotomized rabbits.
Blood iodine of rabbits with ligated bile ducts.

TABLE VI

COMPARISONS OF MEAN VALUES FROM TABLE V

GROUPS OF RABBITS COMPARED	DIFFERENCE OF MEANS	
	PROBABLE ERROR OF DIFFERENCE	
	ERYTHROCYTE COUNTS	LEUCOCYTE COUNTS
A and B (pre- and postop. controls)	6.96	5.50
A and C (preop. controls and ligated 1-10 days)	− 5.62	+ 4.51
A and E (preop. controls and laparotomized 11-27 days)	− 4.45	+ 4.09
B and C (postop. controls and ligated 1-10 days)	− 1.32	+ 5.85
B and D (postop. controls and laparotomized 1-10 days)	− 1.46	+ 4.88
B and E (postop. controls and laparotomized 11-27 days)	− 0.02	+ 0.02
C and D (ligated animals and laparotomized animals 1-10 days)	0.15	3.62
D and E (laparotomized animals 1-10 days and 11-27 days)	1.20	4.29

TABLE VII

GROUP OF RABBITS	DAYS AFTER OPERATION	MEAN VALUE WITH PROBABLE ERROR AND STANDARD DEVIATION OF POLYMORPHONUCLEAR LEUCOCYTE COUNT	DIFFERENCE OF MEANS
			PROBABLE ERROR OF DIFFERENCE
B Postop. controls	1-27	1974±164 ± 1260	
C Ligated animals	1-10	7338±852 ± 5201	
D Laparotomized animals	1-10	3750±380 ± 2497	
B and C (postop. controls and ligated 1-10 days)			+ 6.19
B and D (postop. controls and laparotomized 1-10 days)			+ 4.29
C and D (ligated animals and laparotomized animals 1-10 days)			2.77

TABLE VIII

GROUP OF RABBITS	DAYS AFTER OPERATION	MEAN VALUE WITH PROBABLE ERROR AND STANDARD DEVIATION OF MONOCYTE COUNT	DIFFERENCE OF MEANS
			PROBABLE ERROR OF DIFFERENCE
B Postop. controls	1-27	197±17 ± 131	
C Ligated animals	1-10	376±37 ± 227	
D Laparotomized animals	1-10	311±36 ± 247	
E Laparotomized animals	11-27	175±18 ± 91	
B and C (postop. controls and ligated 1-10 days)			+ 4.39
B and D (postop. controls and laparotomized 1-10 days)			+ 2.87
B and E (postop. controls and laparotomized 11-27 days)			− 0.89
C and D (ligated animals and laparotomized animals 1-10 days)			1.25

b. *Blood Counts.*—The effects of the experimental procedures on the blood counts are shown in Fig. 1 and examined statistically in Tables V, VI, VII, and VIII. The results show that the erythrocyte and leucocyte counts of the postoperative controls were statistically significantly lower than those of the preoperative controls. The erythrocyte counts of rabbits with ligated and divided ducts, of laparotomized rabbits and of their postoperative controls showed no statistically significant differences. Rabbits with ligated and divided ducts and those laparotomized had leucocyte counts significantly higher than their controls for the first ten postoperative days, but not significantly different from each other. The counts returned to within the controls' limits after ten days in the laparotomized rabbits. The increased leucocyte counts were due primarily to increase in the polymorphonuclear leucocytes as shown in Fig. 1 and Table VII. The monocytes were also significantly increased for the ten days in animals with ligated and divided ducts, but not in laparotomized animals as may be seen in Table VIII and in Fig. 1. Lymphocytes, basophiles, and eosinophiles showed no significant changes.

c. *Other Observations.*—The body weight of the animals was followed during the experiment and the findings are given in Table IX. All the rabbits lost weight for the first few weeks.

TABLE IX

AVERAGE BODY WEIGHT OF RABBITS

DATE	NO. OF RABBITS INCLUDED IN AVERAGE	AVERAGE WEIGHT IN KG.
7/22	23	2.61
7/29	23	2.42
8/ 5	23	2.31
8/12	20	2.05
8/19	18	1.97
8/26	18	1.87
9/ 2	15	1.90
9/ 9	15	1.78
9/16	13	1.79
9/23	12	1.85
9/30	10	1.97
10/ 7	10	2.05
10/14	3	1.71
10/21	3	1.75
10/28	3	1.61

Food was analyzed for iodine in the following manner: Two hundred pounds of chopped alfalfa hay were mixed and sampled. Portions of 2.00 gm. of the sample were analyzed for iodine. The average of two analyses showed 10 gamma of iodine per 100 gm. of hay. Three lots of 3 bushels each of oats were mixed separately and similarly sampled and analyzed. The first lot contained 1 gamma of iodine per 100 gm.; the second lot, 6 gamma; and the third lot, 2 gamma. Two lots of fresh cabbage were shredded and 10.0 or 16.0 gm. samples analyzed. The cabbage contained 1 gamma of iodine per 100 gm. in each instance.

Food iodine consumed by the rabbits varied from 0 to 7 gamma (average 3 gamma) per day per rabbit from July 22 to September 10. On September 10 the animals had 11 to 15 gamma (average 13 gamma) and from then to October 7, 0 to 11 gamma (average 7 gamma), after which they had 0 to 5 gamma (average 3 gamma) per day per rabbit to October 30. After October 7 only rabbits 17, 18, and 19 were kept.

Operated animals and their controls were not fed for a day preceding operation and one or two days afterward.

Blood iodine showed neither positive nor negative correlation of appreciable extent with food iodine of the same day or of the preceding day.

Six cans of ether were used during the experiment. Ten milliliters from each can were mixed in an iodine determination flask with one 5 ml. portion of approximately molar KOH and evaporated in a warm water-bath with shaking. Iodine determination was carried out on the residue as with blood. The average of two analyses was 0.1 gamma of iodine per 60 ml. of ether.

Rabbits with ligated and divided ducts showed extensive macroscopic liver damage on autopsy. Even in rabbit 13, which died fifteen hours after ligation, the lower right lobe of the liver was almost completely yellow. Only rabbit 7 showed jaundice (twelve days after ligation). Rabbit 17 failed to show jaundice, although he survived ligation for twenty-four days. On autopsy he was found to have enlarged bile ducts and an enlarged gall bladder, but the liver showed no macroscopic changes beyond several tiny yellow spots.

Thyroids from control rabbits 9, 22, and 23 showed hypertrophy and small areas of hyperplasia, and in 9 and 23, some diminution in colloid substance. Thyroids from rabbits 4 and 7 (with ligated bile ducts) also showed small areas of hyperplasia. Colloid distended the acini in rabbits 4 and 13 (with ligated bile ducts), but was absent from thyroids of rabbits 7 and 10 (with ligated bile ducts). It was also absent in the laparotomized rabbit 11, while rabbit 24 showed focal loss of colloid. These were the only tissues examined histologically.

Results on rabbits 24 through 30 were not included in the calculations because of their unusually high initial blood iodine values as compared with the other animals. These rabbits were not operated or used as controls. Results on rabbit 17 after operation were also omitted from calculations, since his common bile duct was not divided as were those of the other six ligated rabbits.

DISCUSSION

The normal blood iodine of rabbits 1 through 23 was 3 to 5 gamma per 100 ml. as compared to Hercus and Aitken's[10] figures of 60 to 80 gamma per kg., the figures of Magee and his associates[12] of 8.6 to 9.8 gamma per 100 c.c., the average figure of Elmer and co-workers[8] of 13.2 gamma per 100 c.c., and the figures of Anderson and others[1] of 10 to 15 gamma per 100 c.c. Whether these differences are due to different amounts of iodine in the blood or to the erroneously high values obtained by practically all of the earlier workers (as

Baumann and Metzger[2] have pointed out), we do not know. We are also unable to account for the high blood iodine values of rabbits 24 through 30, even after some days of maintenance on food containing only a few gamma of iodine per day.

The blood iodine increased during the ten days after operation under ether anesthesia whether the operation included ligation and division of the common bile duct or was merely an abdominal incision. After ten days the laparotomized rabbits had a normal blood iodine, while most of the rabbits with ligated bile ducts were dead and the few which survived longer had 4 to 7 gamma of iodine per 100 ml. of blood. Others have also noted an elevated blood iodine following various surgical procedures,[3, 4, 11] but they have failed to discuss the possible relation of the blood iodine to the anesthetics used in these instances. Hereus and Aitken[10] found a decrease in blood iodine of one rabbit after twenty minutes of ether anesthesia and an increase in a second rabbit after sixty minutes of ether anesthesia. But Magee and co-workers[12] could find no change in blood iodine in sixteen rabbits under ether anesthesia.

The difference in erythrocyte and leucocyte counts of pre- and postoperative controls may have been due to increased withdrawal of blood or to the diet and the heat of their living quarters, which were over 30° C. on most days. Operation with and without common bile duct ligation significantly increased the leucocyte and polymorphonuclear leucocyte counts over the controls' counts for a ten-day period. Monocytes were also significantly increased only in animals with ligated bile ducts. In this connection it is of interest to note that Mills and others[13] wrote that common bile duct obstruction should produce constant and progressive blockade of the reticulo-endothelial system, of which the monocytes are a part. Contrary to Zih's report[19] that erythrocytes decrease 23 per cent after occlusion of the common bile duct in rabbits for four days, we found no statistically significant change in erythrocyte counts on operation with or without ligation and division of the common bile duct.

Although statistically significant differences have been shown among certain of these blood counts, the mean values are within the widely varying normal limits of rabbits' counts as given in the literature.[9]

CONCLUSIONS

1. Ligation and division of the common bile duct of male rabbits significantly increased their blood iodine, their leucocyte, polymorphonuclear leucocyte, and monocyte counts, but did not significantly change their erythrocyte, lymphocyte, basophile, or eosinophile counts for a ten-day period after operation.

2. Abdominal incision of male rabbits significantly increased their blood iodine, their leucocyte and polymorphonuclear leucocyte counts, but did not significantly change their erythrocyte, lymphocyte, monocyte, basophile, or eosinophile counts for a ten-day period after operation.

3. While the differences in blood iodine and blood counts between male rabbits for a period of ten days after abdominal incision with or without liga-

tion and division of the common bile duct were not statistically significant, the duration of the effect of operation in animals subjected to abdominal incision only was about ten days.

4. Male rabbits with ligated and divided common bile ducts died within thirteen days after operation.

We wish to acknowledge the assistance of Miss Mary Rita Moormann with the blood counts, the advice of Dr. Willard Machle of the Kettering Laboratory on the statistical analyses, the histological study of Dr. M. Ganim and Dr. J. Ganim, and the suggestions of Dr. I. A. Mirsky.

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A CYTOLOGIC COMPARISON OF MALIGNANT AND NONMALIGNANT NUCLEI AND NUCLEOLI*

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THE present study is a continuation of the work begun in 1933.[3, 9, 10] The purpose is to demonstrate and to emphasize the fact originally stressed, that nucleoli in malignant cells are enlarged to such an extent that they are readily distinguishable from nucleoli of all other types of cells.

Several years ago, MacCarty[5-7] observed and called attention to enlargement of nucleoli in malignant cells. Other authors have studied these structures in cells of exudates and transudates.[4, 13, 14] Their results can be summarized briefly as follows: "The increase in nucleolar size in relation to the nuclei is characteristic for the cell of malignant neoplasms found in transudates and exudates." The results of several recent investigations[11, 12, 15, 17] are in accord on one point, that nucleoli of malignant cells are changed in such a way that in every microscopic field one finds many, if not all of them, enlarged

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to an extent never observed in nonmalignant cells; therefore, the enlarged nucleolus can be designated as characteristic of malignant cells.

In distinction to the cellulonuclear ratio[18] which is said to be fairly constant for each type of cell, one finds the nucleonucleolar ratio of malignant cells definitely lower.

This paper presents a résumé of nuclear and nucleolar measurements of various tissues obtained in 152 cases, in the majority of which malignancy was present. The method of measuring and of computing the area of these structures is very similar to that described in the first publication.[19] The

TABLE I

AVERAGE AREAS OF NUCLEI AND NUCLEOLI IN MALIGNANT AND NONMALIGNANT TISSUES

TISSUE	CASES	AVERAGE AREA, SQUARE MICRONS		AVERAGE RATIO
		NUCLEUS	NUCLEOLUS	
Breast				
Carcinoma	54	80.2	6.1	13.1
Secondary cytoplasia	2	65.5	4.6	14.2
Inflammatory	9	48.5	2.1	23.1
Stomach				
Carcinoma	16	78.3	4.6	17.0
Lymphosarcoma	3	67.7	5.2	13.0
Secondary cytoplasia	7	75.2	4.1	18.3
Regenerative	6	58.9	1.8	32.7
Intestine				
Carcinoma	17	71.1	5.3	13.4
Regenerative	5	52.9	1.8	29.3
Uterus				
Carcinoma	6	65.8	4.1	16.0
Sarcoma	2	83.9	5.1	16.5
Hyperplastic endometrium	2	45.1	1.4	32.2
Ovary				
Carcinoma	9	76.4	4.8	15.9
Oophoritis	3	59.6	1.4	42.6
Kidney				
Hypernephroma	8	87.3	6.8	12.8
Epithelioma (ureter)	1	119.1	8.9	13.4
Nonmalignant	2	52.9	2.8	18.9
Thyroid				
Carcinoma	1	71.5	5.1	14.0
Adenoma	1	45.4	1.8	25.2
Skin				
Epithelioma	3	64.4	4.9	13.1
Nonmalignant	2	56.2	2.1	26.8

results obtained by examination of only freshly cut and frozen tissues (not imbedded tissues) are illustrated in the tables. It was found by experience that the coagulation of albuminoid constituents of the cell by various fixatives causes considerable shrinkage of tissue to the extent of approximately 10 per cent.[1]

During investigation of structures of cells, interesting observations were made; it was noteworthy that some similarity existed between the structures, even in so-called lawless growths. The nuclei and nucleoli of cells of carcinoma of the breast showed more similarity in size when compared with each

other than when compared with cells of carcinoma of the stomach, and vice versa. In measuring twenty to thirty cells in each section of tissue and by studying a large number of specimens one obtains much better insight into sizes and variations of nuclei and nucleoli than by merely being presented with an average value for a large number of cells and cases, as one has to do in a short presentation. Comparison of fluctuations in nuclear, and particularly in nucleolar, sizes in malignant and nonmalignant cells in a large number of these structures is most interesting and instructive. Increase particularly in size of nucleoli in cells of carcinomatous tissue is most striking. Unfortunately all these measurements cannot be presented in this report, and average values must be given in Table I.

Table I represents average sizes of nuclei and nucleoli of various tissues of the body, as well as average ratios of area of nuclei to area of nucleoli. It is seen that areas of nuclei and nucleoli are much larger in malignant cells than in nonmalignant ones; of these, the sizes of nucleoli are also much larger than the sizes of nuclei; the ratios are markedly smaller for malignant cells than for nonmalignant cells. The cases of secondary cytoplasia, a condition described by MacCarty,[8] are added because it is interesting to observe that areas of nuclei and of nucleoli in such lesions approach very nearly, and sometimes even attain, the same values that are found for areas of malignant cells. These cells described by MacCarty, however, are found in situ. It is felt that if these benign cells were left in an opportune environment, the danger of their becoming malignant would be imminent.

Fig. 1 represents the mean area of largest and smallest nuclei and nucleoli expressed graphically. Under the heading "nuclei," curves 1 and 3 designate largest and smallest areas of malignant cells, and curves 2 and 4 designate largest and smallest areas in nonmalignant cells. Corresponding curves for nucleoli are illustrated. Whereas the curves for nuclei of malignant and nonmalignant cells approach or even cross one another, the curves for nucleoli are far apart.

Fig. 2 is a graphic comparison of a group of lesions of the mammary gland comprising 42 cases in which malignant and nonmalignant conditions were present. The mean area for both nuclei and nucleoli is larger in malignant lesions than in nonmalignant ones. In the percentage distribution the curves for nuclear values of both malignant and nonmalignant lesions illustrate some overlapping, which means that the same sizes or nearly the same sizes are occasionally found in either lesion. The curves for nucleoli, however, illustrate hardly any overlapping, which means that nucleoli in malignant lesions are definitely enlarged when compared with nucleoli of nonmalignant ones. This confirms the contention that nucleoli are increased in size in malignant cells.

Figs. 3 and 4 are drawings of nuclei and nucleoli seen in cells respectively of the stomach and of the kidney. The nuclei and nucleoli were drawn with the aid of a camera lucida. The nucleoli were again observed to be enlarged to measurable sizes and are illustrated in the lower part of the drawing. The sizes in malignant and nonmalignant lesions, particularly of the nucleoli, illustrate distinct changes.

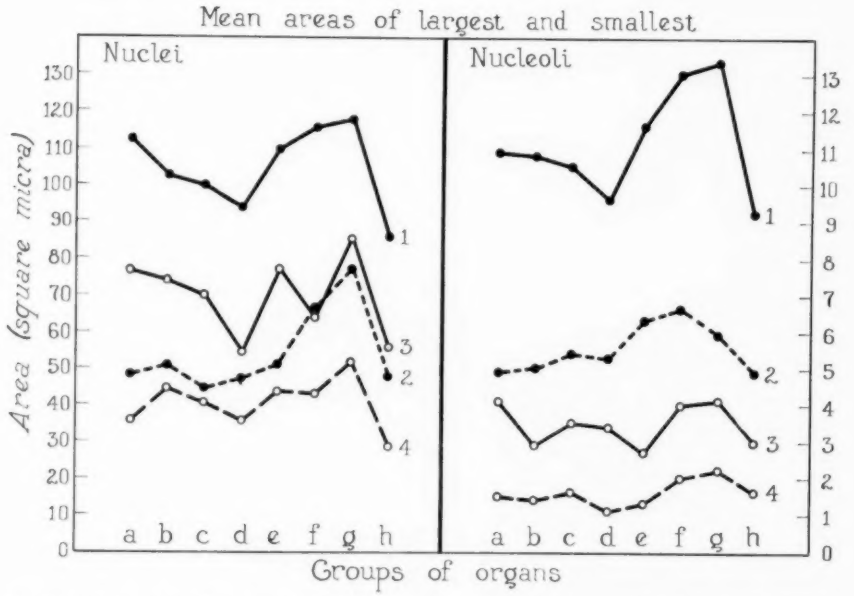

Fig. 1.—Summary of study of malignant cells from various organs: *a*, breast; *b*, stomach; *c*, intestinal tract; *d*, uterus; *e*, ovary; *f*, kidney; *g*, ureter; *h*, skin.

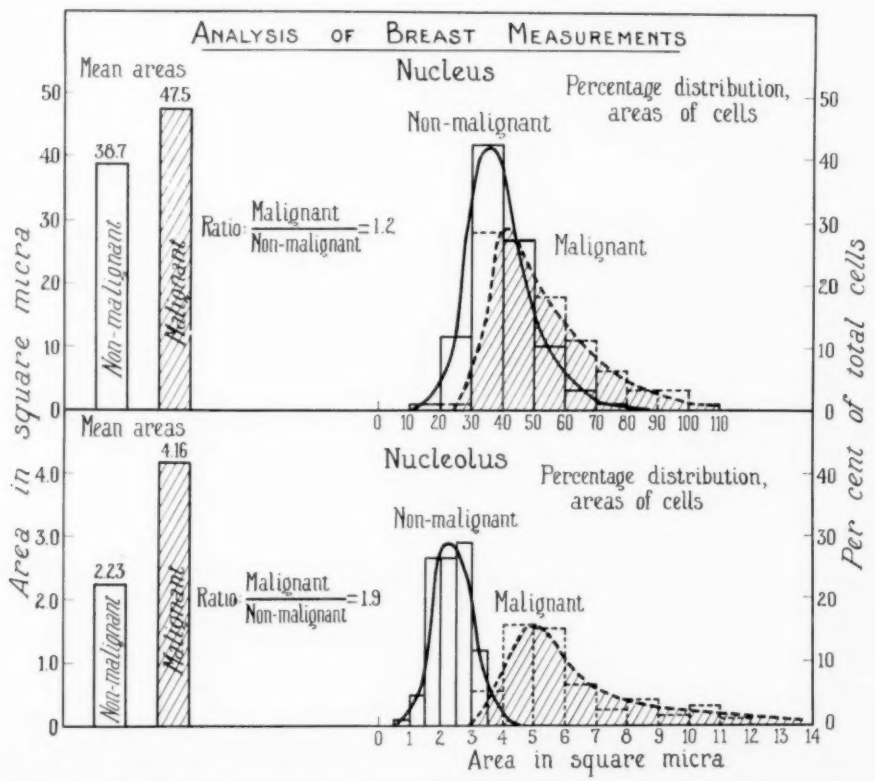

Fig. 2.—Comparison of measurements of nuclei and nucleoli of malignant and nonmalignant cells of the mammary gland.

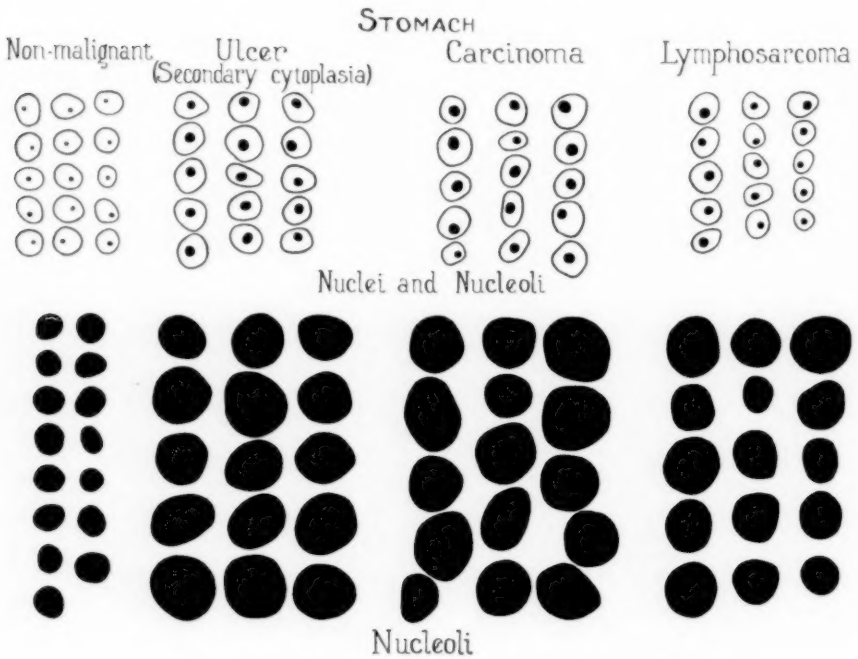

Fig. 3.—Nuclei and nucleoli of cells of the stomach.

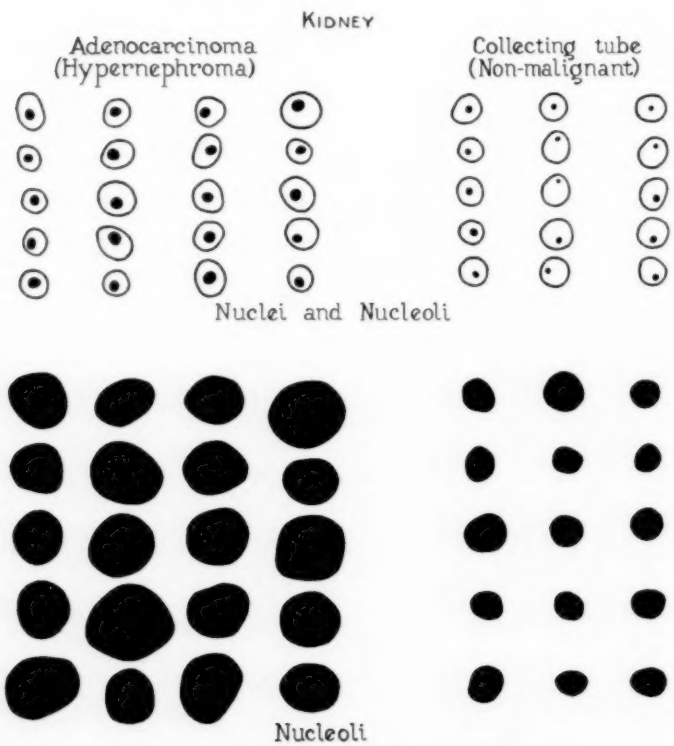

Fig. 4.—Nuclei and nucleoli of cells of the kidney.

Exceptions to these observations have been found in the so-called comedocarcinomas. Hardly any nucleoli could be seen in these cells and, if present, they were not enlarged markedly. Some leading authorities, however, do not regard this as a malignant lesion.

The reason for increase in size of nucleoli in malignant cells is not known, nor is the function of nucleoli sufficiently understood. Nucleoli are considered by some authors to be by-products of nuclear activity[2] which are derived from the chromatin; others[16] consider nucleoli storage places of kinoplasm, a material from which the spindle fibers are built during mitosis. Whatever their function or purpose may be, increase in size of nucleoli in malignant cells is certainly worth attention. Enlargement of nucleoli takes place at an early stage in the changing cell as can be observed in cases of secondary cytoplasia.

SUMMARY

Nuclear and nucleolar sizes have been studied and measured in 152 cases, representing various lesions throughout the body. The table and figures illustrate that nuclei, and particularly nucleoli, in malignant cells are larger than in corresponding nonmalignant cells. Graphs illustrate the same results. It is, therefore, justifiable to designate an enlarged nucleolus as a characteristic of malignant cells. As it is found at an early stage in the changing cell and is easily observed in these cells, the enlarged nucleolus should become one of the most helpful means of diagnosis of malignant cells.

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SUPERIMPOSED INFECTION IN RHEUMATIC HEART DISEASE*

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SUBACUTE bacterial endocarditis has generally been considered a superimposed infection when it occurs in cases of rheumatic heart disease. An alternative hypothesis[1] suggests that it represents the terminal phase of an infection in which rheumatic valvulitis is the allergic component. This implies that when bacterial endocarditis appears, immunity has broken down. Pending the elucidation of the bacteriology of rheumatic fever, the term "superimposed" infection must be used with the reservation required by the allergic hypothesis.

Subacute bacterial endocarditis is not the only superimposed infection found in rheumatic heart disease nor is it the most frequent. In a series of 30 cases, selected as representative of the latter disease as seen in a general hospital, evidence of superimposed infection could be demonstrated in the heart in 22 cases. Of these, only one had the clinical course of subacute bacterial endocarditis. The others included acute bacterial endocarditis, septicemia, erysipelas, pneumonia, and peritonitis. In most of these cases, acute inflammatory, nonrheumatic lesions were found in the myocardium. Many also had acute endocarditis which was neither bacterial nor rheumatic. This has been described as "indeterminate,"[2] "terminal" and "non-bacterial thrombotic" endocarditis.[3] Its frequent association with acute nonrheumatic myocarditis, the features of which will be described below, suggests that these endocarditides should be included as one of the cardiac sequelae of superimposed infection.

The cases presented in this study are classified as follows:

	ACUTE ACTIVE RHEUMATIC CARDITIS	CHRONIC ACTIVE RHEUMATIC CARDITIS	HEALED RHEUMATIC CARDITIS	TOTAL
A. Without significant superimposed infection	3	3	2	8
B. With slight to severe superimposed infection	5	6	2	13
C. With predominant superimposed infection	2	5	2	9
Total	10	14	6	30

CASE REPORTS

A. Without Significant Superimposed Infection

CASE 1.—Negress, aged 38 years, had scarlet fever in childhood. Rheumatic fever at 37 years, hospitalized for one month. Subsequent symptomatology was that of decompensation, at first slight, later with massive edema. Systolic and presystolic murmurs at apex,

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systolic murmur at base. Blood pressure 96/62 and 132/110 mm. Hg. Electrocardiogram: low voltage QRS waves. Roentgenogram: decompensated mitral stenosis; pleural effusion. Low grade febrile course for six months with leucocytosis. Six blood cultures, the last one ten days before death, negative Two cultures six months before death contained staphyloid organisms. Death during an attack of paroxysmal dyspnea.

Pathology.—Acute rheumatic valvulitis of one aortic cusp; healed in others. Healed rheumatic mitral valvulitis (stenosis). Acute rheumatic myocarditis showing extraordinary numbers of Aschoff bodies. No superimposed infection.

CASE 2.—Male, 38 years of age, admitted suffering from pulmonary edema. Died several hours later.

Pathology.—Chronic active rheumatic valvulitis of aortic and mitral valves (stenosis). Acute active rheumatic myocarditis. No superimposed infection.

CASE 3.—Negress, 38 years old, had polyarthritis at 32 years; repeated two years later and intermittently thereafter. Joint lesions of arthritis deformans type complicated by scleroderma and bone atrophy. Blood calcium 7 mg., phosphorus 6 mg. Systolic and diastolic murmurs at apex. Roentgenogram: contour not distinctive of mitral stenosis. Electrocardiogram: low voltage QRS waves; A-V conduction time 0.20 to 0.32 second. Temperature level fluctuated intermittently, rising occasionally to 102.8° F. Terminally, developed right inguinal abscess.

Pathology.—Acute and chronic active rheumatic pancarditis. Adhesive pericarditis. Aschoff bodies in left ventricle and interventricular wall only. Lymphoid, plasma and eosinophilic cell infiltration in myocardium.

CASE 4.—Negro, aged 17 years, had polyarthritis, chorea, and carditis at 8 years. Relatively slight disability in following nine years, symptoms being joint pains and dyspnea. Three weeks before admission he had an upper respiratory infection, blood-streaked sputum and became increasingly dyspneic. Systolic and diastolic murmurs at apex; diastolic aortic murmur. Blood pressure 140/0 and 240/0 mm. Hg. Pistol shot sounds and Corrigan pulse. Roentgenogram: tremendous enlargement of heart, obscured contours suggesting effusion. Electrocardiogram: auricular fibrillation, left axis deviation. The course was febrile, up to 104° F., congestive failure was always present. Precordial pain was a very troublesome feature. Three blood cultures were negative. On the eleventh day, a convulsive seizure was followed by deep stupor. This was repeated several times. Death occurred five days after first attack.

Pathology.—Chronic active rheumatic valvulitis of all valves. Acute active rheumatic myocarditis, infrequent Aschoff bodies. Acute fibrinous and chronic adhesive pericarditis. Granular degeneration of myocardial fibers without cellular reaction. Acute encephalitis. Acute focal glomerulitis. Chronic pneumonitis.

CASE 5.—White female, aged 23 years, had rheumatic fever at 14 years and three recurrences. At 21 years she had an induced abortion in the fifth month. Admitted in the ninth month of second pregnancy, giving history of dyspnea since third month and an upper respiratory infection for several days. Afebrile on admission. Cardiac status, mitral stenosis. Blood Wassermann, four-plus; untreated. Normal delivery on fifth day. Pulmonary edema four hours later; death in eight hours.

Pathology.—Chronic active rheumatic valvulitis and myocarditis. Granular degeneration of myocardium without cellular reaction. Many gram-negative bacilli in myocardium, probably agonal. Syphilitic aortitis. Acute pneumonitis.

CASE 6.—White woman, aged 40 years, gave no rheumatic history. Right hemiplegia and aphasia at 38 years. Chief symptom, dyspnea. Second hemiplegia on evening of admission. Mitral stenosis and auricular fibrillation. Blood pressure 140/70 mm. Hg. Four weeks later convulsions, unconsciousness. Terminal pneumonia. Death in five days. Electrocardiogram: auricular fibrillation; low voltage QRS waves.

Pathology.—Healed rheumatic valvulitis of mitral, aortic, and tricuspid valves. Chronic active rheumatic myocarditis. Myocardial granular degeneration without cellular reaction. Recent multiple cortical hemorrhages. Acute pneumonitis.

CASE 7.—White male, aged 61 years, had rheumatic fever at 23 years. Frequent sore throats. Admitted in cardiac failure; duration of symptoms five years. Mitral stenosis. Blood pressure 118/68 mm. Hg. Congested lung bases and enlarged liver. Electrocardiogram: auricular fibrillation, low voltage QRS waves. Roentgenogram: mitral stenosis and left ventricular enlargement. Discharged and readmitted two weeks later in congestive failure, temperature 103° F. Right lower lobe pneumonia and pleurisy.

Pathology.—Healed rheumatic aortic and mitral valvulitis (stenosis). Healed rheumatic myocarditis. Granular degeneration of myocardial fibers without cellular reaction. Acute bronchopneumonia and pleurisy.

CASE 8.—White woman, 63 years old, suffered from chronic arthritis for fifteen years. Dyspnea and leg edema, three months. Systolic and diastolic murmurs at apex. Blood pressure 210/80 mm. Hg. Congestive failure, cyanosis, hydrothorax. Electrocardiogram: auricular fibrillation, premature ventricular contractions. Death four days after admission.

Pathology.—Healed rheumatic mitral and aortic valvulitis. Healed rheumatic myocarditis. Granular degeneration of myocardial fibers without cellular reaction. Acute pneumonitis.

DISCUSSION

These cases illustrate the natural history of rheumatic heart disease uncomplicated by significant superimposed infection. Three cases had acute rheumatic carditis, i.e., characteristic valvulitis and Aschoff bodies in the myocardium. Three cases had chronic inflammatory lesions in the myocardium and ring regions. In two cases, the rheumatic lesions were healed, the valves being distorted and sclerosed and myocardial and ring scars having little or no cellular reaction.

Six cases had infections which are not generally considered rheumatic. Three had pneumonitis, one pneumonitis and pleurisy, one pneumonitis and encephalitis, and one had an inguinal abscess. Despite these infections, the myocardial pathology was limited to rheumatic lesions, although five of the cases had granular degeneration of the muscle fibers (Fig. 1). In the absence of cellular reaction this was not considered evidence of significant superimposed infection. Case 6 also had mural endocarditis, but there were fresh vegetations on the valves in only one other case (Case 8), and fibrinous pericarditis in only two cases (Cases 3 and 4).

CASE REPORTS

B. With Slight to Severe Superimposed Infection

CASE 9.—Japanese boy, 3 years old, gave history of upper respiratory infection of two weeks' duration and moderately severe abdominal pain. Bronchopneumonia at right lung base. Low grade febrile course, 99° to 102° F., brief rises to 103° F. Leucocytosis, 13,800 to 20,200 per c.mm.; highest polymorphonuclear count, 81 per cent. Loud systolic murmur over precordium and left axilla. Second pulmonic sound very loud. Roentgenogram: enlarged left ventricle, prominent pulmonic conus, no definite consolidation of lung. Electrocardiogram: simple tachycardia, flat T-waves in Lead I. Two blood cultures were negative. Nine days before death, a blood culture was positive for hemolytic *Staphylococcus albus*. Total duration of illness, six weeks.

Pathology.—Acute active rheumatic pancarditis. Acute fibrinopurulent pericarditis. Widespread myocardial necrosis and polymorphonuclear infiltration. Acute bronchopneumonia. Superimposed infection: staphylococcal bacteriemia.

CASE 10.—White boy, aged 10 years, had scarlet fever three months before death. In second week of scarlet fever, cervical adenitis and polyarthritis; in fifth week, presystolic apical murmur. Roentgenogram: bronchopneumonia and pericardial effusion. Fluid and blood cultures were negative. Febrile course; leucocytosis up to 11,300 per c.mm. Electrocardiogram: right axis deviation, flat T-waves in Leads I and II. Blood culture ten days ante mortem contained streptococci, type not determined.

Pathology.—Acute active rheumatic pancarditis. Acute endocarditis of mitral, aortic, and tricuspid valves, and vegetations on the mural endocardium. Acute myocardial abscesses. Acute fibrinous pericarditis. Epicardial abscesses. Acute broncho- and interstitial pneumonia. Acute rheumatic aortitis. Superimposed infection: streptococcal bacteriemia.

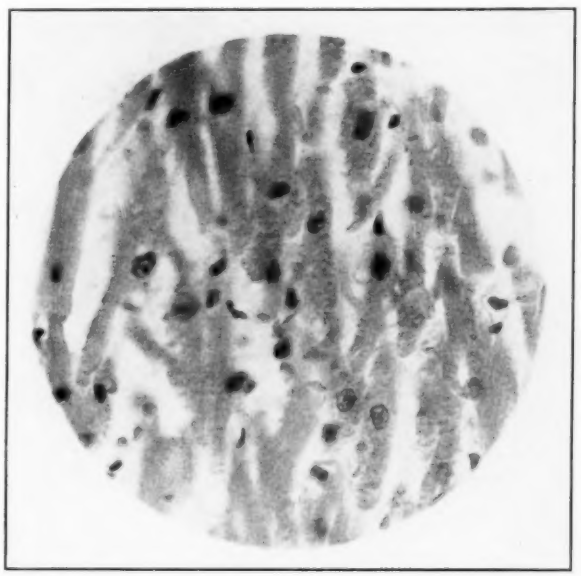

Fig. 1.—Molecular degeneration of the myocardium found in cases without significant superimposed infection. The fibers are granular, the cross striations are coarsened and frequently replaced by an amorphous granular mass. One degenerating polynuclear cell is present (Case 5).

CASE 11.—Negro, 11 years old, gave no history of rheumatic infection. Few upper respiratory infections. Three weeks before admission, had "cold," pleurisy, and pneumonia. Tachycardia and orthopnea, but not febrile in hospital. Systolic and diastolic apical and diastolic aortic murmurs. Within a few days, he became restless, and had precordial and abdominal pain. Death occurred six days after admission. Temperature never above 100° F. Pulse always rapid, 100 to 132 per minute.

Pathology.—Acute active rheumatic pancarditis. Acute fibrinous pericarditis. Myocardial abscesses. Superimposed infection: acute bronchopneumonia.

CASE 12.—White male, aged 17 years, had rheumatic fever at 12 years; in bed seven months. Second bout at 14 years, in bed three months. On admission at 15 years, had dyspnea on slight exertion and precordial pain radiating to the left shoulder. Presystolic apical thrill and murmur, aortic systolic murmur. Hospitalized two years and eight months. Almost continuous slight elevation of temperature, generally about 100° F., short episodes to 101° F. or higher, occasionally normal for a few weeks. Repeated blood cultures

negative. Electrocardiogram: right axis deviation and isoelectric T-waves in some tracings only; conduction time variable but never above 0.20 second. Roentgenogram: mitral stenosis contour. Died suddenly.

Pathology.—Acute and chronic active rheumatic pancarditis. Acute myocardial necrotic foci infiltrated by polymorphonuclear cells. Adhesive and fibrinopurulent pericarditis. Superimposed infection: undetermined origin.

CASE 13.—Negress, aged 29 years, had rheumatic fever at 8 years. Repeated hospitalizations in following twenty years for rheumatic heart disease. On admission, she had been in congestive failure for three months. Febrile. Mitral stenosis. Electrocardiogram: low voltage QRS waves. Roentgenogram: aortic and mitral stenosis. Blood culture negative. Predominant clinical features were mechanical failure and infection, presumably rheumatic.

Pathology.—Acute and chronic active rheumatic pancarditis. Many foci of acute myocardial abscesses. Superimposed infection: type and source undetermined.

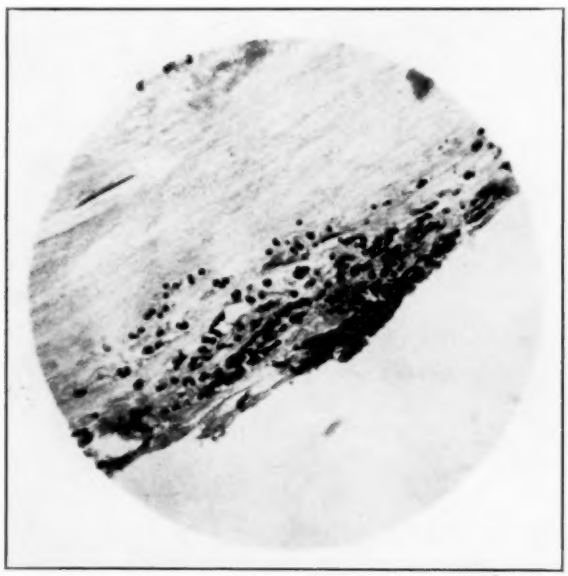

Fig. 2.—Acute endocarditis of the mitral valve (Case 19).

CASE 14.—White male, 35 years old, was admitted at age 32 years, after six months of partial disability due to dyspnea and precordial pain. Rheumatic fever at 24 years. Irregular febrile course for three months slowly subsiding until discharge. Systolic and diastolic apical and basal murmurs, aortic diastolic murmur of variable audibility.

Subsequently he was readmitted to the hospital five times, the status and course during each admission being similar, i.e., slowly subsiding fever. Repeated blood cultures during all admissions were negative with the exception of one, which grew an indifferent streptococcus. The agglutination titer to this organism increased with improvement. Throat cultures contained *Streptococcus viridans* and *hemolyticus*. Sedimentation rates and blood counts fluctuated with the fall and rise of infection. Roentgenogram: pulmonary fibrosis and stasis; mitral stenosis and failure. Electrocardiogram: left axis deviation; T-waves upright when not digitalized; conduction time 0.20 to 0.24 second, without digitalis; under digitalis as high as 0.30 second.

Pathology.—Chronic active rheumatic valvulitis of mitral, aortic, and tricuspid valves. Chronic active rheumatic myocarditis. Numerous foci of acute myocardial degeneration infiltrated by polymorphonuclear cells. Superimposed infection: type and origin undetermined.

CASE 15.—White female, aged 37 years, admitted in congestive failure; history unobtainable. Systolic and diastolic apical murmurs. Blood pressure 195/125 and 205/130 mm. Hg. Hemorrhages and exudate in fundi. Roentgenogram: diffuse cardiac enlargement, pulmonary stasis, pleural effusion. Death twenty-four hours after admission.

Pathology.—Chronic active rheumatic pancarditis; mitral stenosis. Granular myocardial degeneration and polymorphonuclear infiltration. Superimposed infection: type undetermined.

CASE 16.—White male, 41 years old, had "rheumatism" at 25 years; pleurisy at 40 years, subsequently severe hemoptysis, dyspnea, and slight leg edema. For four weeks before admission, night sweats. On admission, slightly febrile, subsiding after a few days, and remaining normal for two weeks. Moderate congestive failure. Systolic and diastolic apical murmurs, systolic aortic murmur. Blood pressure 130/80 mm. Hg. Electrocardiogram: low voltage QRS waves, premature ventricular contractions, right axis deviation. Roentgenogram: mitral stenosis, pleural thickening. In the third week, temperature rose and petechiae of conjuctivae and chest appeared. Pleuropericardial friction rub. Four blood

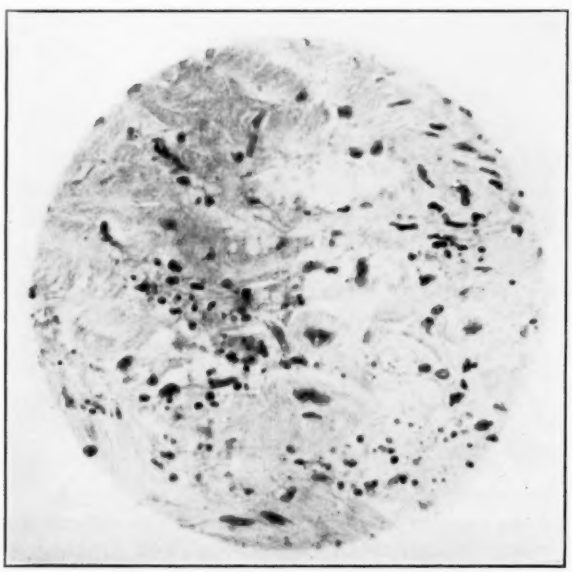

Fig. 3.—Acute degenerative lesion with polymorphonuclear cell infiltration and demonstrable cocci within the area, interpreted as early miliary abscess (Case 29).

cultures negative, one contained staphyloid organisms. Death after febrile course of seven days, signs of congestive failure and infection increasing rapidly.

Pathology.—Chronic active and healed rheumatic pancarditis; mitral stenosis. Acute endocarditis of mitral, aortic, and tricuspid valves, and vegetations on mural endocardium. Fibrinous and adhesive pericarditis. Foci of subendocardial polymorphonuclear cells. Superimposed infection: pneumonia and staphylococcal bacteriemia.

CASE 17.—White male, 46 years of age, had scarlet fever in childhood. Rheumatic fever at 12 and 32 years. Numerous attacks of grippe, influenza, and bronchopneumonia. For two years, increasing dyspnea, fatigue, and gastrointestinal symptoms. Painful bleeding hemorrhoids for several years. On admission, mild congestive failure. Mitral stenosis; diastolic tricuspid murmur. Electrocardiogram: auricular fibrillation; right axis deviation. Roentgenogram: mitral and aortic valvular disease; pulmonary congestion. After compensation improved, hemorrhoidectomy under spinal anesthesia was performed. Death twelve hours later, the clinical status indicated vasomotor collapse and congestive failure.

Pathology.—Chronic active rheumatic valvulitis and myocarditis; acute fibrinous pericarditis. Acute myocardial degeneration infiltrated by many polymorphonuclear cells. Multiple

mural thrombi of pulmonary arteries. Acute infarctions of lungs and kidneys. Acute focal glomerulitis. Superimposed infection probably infected hemorrhoids.

CASE 18.—Male, white, aged 59 years, had polyarthritis at 34 years; syphilis and gonorrhea at 32 years. The cardiac symptoms which began one and one-half years before admission were dyspnea and, later, leg edema. He was in the hospital for two weeks and improved. Admitted finally in congestive failure with massive edema. Systolic apical murmur. Blood pressure 150/60 mm. Hg. Roentgenogram: cardiac contour not distinctive, suggesting only cardiac failure. Died two days after admission.

Pathology.—Chronic rheumatic valvulitis of mitral and aortic valves. Chronic active rheumatic myocarditis. Areas of muscle necrosis with polymorphonuclear infiltration and a surrounding zone of hemorrhage. Superimposed infection: source undetermined.

CASE 19.—Negress, aged 62 years, admitted for vaginal bleeding. No rheumatic history. Heart negative. Hysterectomy for leiomyosarcoma uteri. On the seventh day postoperative she complained of precordial pain. Auricular fibrillation noted for first time; apex rate 125, radial pulse 83 per minute. Effectively digitalized and appeared to be making a good recovery. Highest temperature level 100.4° F. On the thirteenth postoperative day, temperature 99.4° F. The following day she had severe precordial pain and died suddenly.

Pathology.—Chronic active rheumatic mitral valvulitis. Acute and chronic active rheumatic myocarditis; few Aschoff bodies and many foci of eosinophilic and polymorphonuclear reaction. Acute myocardial abscesses. Acute endocarditis of mitral valve (Fig. 2). Infected surgical wound. Acute focal hepatitis. Superimposed infection at operative site.

CASE 20.—White, male, aged 46 years. No history of rheumatic fever. Dyspnea and recurrent ankle edema for unknown period of years. Last illness began three weeks before death with congestive failure. On admission, he had orthopnea, cyanosis, slight icterus, edema, an enlarged liver, pulmonary congestion, and possibly pneumonia. The heart was enlarged. Rough apical systolic murmur; aortic systolic murmur and thrill and diastolic blow. Blood pressure 85/45 and 140/70 mm. Hg. Electrocardiogram: Late T_1 inversion; notched, slurred, and widened QRS waves; left axis deviation. Temperature rose to 101.8° F. Death sixty hours after admission.

Pathology.—Healed rheumatic valvulitis and myocarditis. Many foci of acute myocardial necrosis, infiltrated with few polymorphonuclear cells. Superimposed infection: acute pneumonitis of right upper and left lower lobes.

CASE 21.—Negress, aged 68 years. No history of rheumatic fever. For several months had precordial pain, palpitation, and dyspnea on exertion. On the morning of admission she had severe abdominal pain, vomited several times, and became very dyspneic. Had three bowel movements. Auricular fibrillation, pulse deficit. Blood pressure 242/120 mm. Hg. Systolic murmurs at apex and base. Abdomen tender and rigid, moderately distended. Slight fecal return from enema. Partial intestinal obstruction due to mesenteric thrombosis was diagnosed, but the patient was "in extremis" and operation impossible. Death thirty hours after admission.

Pathology.—Healed rheumatic valvulitis, mitral, and aortic stenosis. Healed rheumatic myocarditis. Many foci of myocardial degeneration and slight polymorphonuclear infiltration. Superimposed infection: acute peritonitis, origin undetermined.

DISCUSSION

The characteristic feature of this group of cases was the presence in the myocardium of polymorphonuclear reaction in regions of muscle degeneration, forming in many instances miliary abscesses (Figs. 3 and 4), or areas of slight cellular infiltration. In five cases the nature and source of the infection could

not be determined. One of these had a transient streptococcus in the blood stream (Case 14). The other infections were pneumonitis (5 cases), infected hemorrhoids, infection in an operative wound, and acute peritonitis (1 case each). Two cases (Cases 9 and 16), with pneumonitis, had transient staphylococci in the blood. Six cases had fresh vegetations on the valves, one associated with mural endocarditis. Nine had fibrinous or chronic adhesive pericarditis, only one with Aschoff bodies in the epicardium. The incidence of fresh vegetations and pericarditis is definitely higher than in the cases without significant superimposed infection.

The severity of acute myocardial necrosis and miliary abscess formation varied considerably, being greatest in Cases 9 and 10, and least severe in Cases

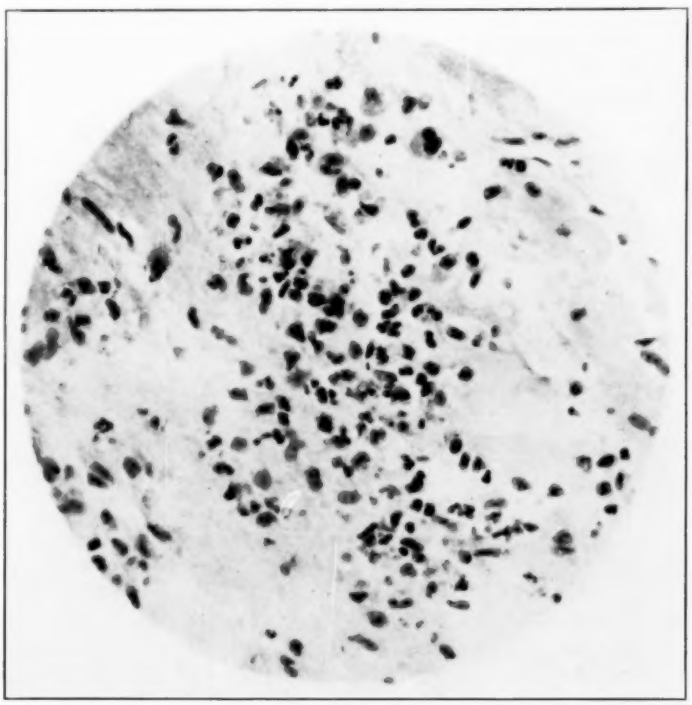

Fig. 4.—Definite well-established miliary abscess (Case 9).

20 and 21. The former were cases of active rheumatic carditis in children, the latter healed rheumatic carditis in the fifth and seventh decades of life. The severity of the nonrheumatic reaction to superimposed infection apparently parallels the severity of the rheumatic myocardial reaction and the age. Case 9, aged 5 years, had a moderately short course, lasting only six weeks; the superimposed infection was pneumonitis and staphylococcal bacteriemia. The myocardial reaction was mixed, consisting of numerous Aschoff bodies and areas of necrosis and myocardial abscess formation. In Case 10, age 10, the course was slightly longer, the superimposed infection streptococcal bacteriemia and pneumonitis. Aschoff bodies were numerous and the polymorphonuclear reaction and miliary abscess formation severe. This is in marked contrast to the mild

reaction in Cases 20 and 21. In these, the ages were in the latter decades of life, the rheumatic infection relatively quiescent, and the superimposed infection of very short duration.

CASE REPORTS

C. With Predominant Superimposed Infection

CASE 22.—White, female, 29 years old, gave no history of rheumatic fever. Two weeks before admission, she was injured in an automobile accident, and a few days later was hospitalized because of dyspnea. She was in the fifth month of pregnancy. Moderate anemia; systolic and presystolic apical murmurs. Electrocardiogram: tachycardia only. Blood culture eleven days before death was positive for hemolytic streptococcus. Hysterotomy was performed. She died two days later.

Pathology.—Acute and chronic active rheumatic pancarditis with deformed mitral and aortic cusps; acute endocarditis of mitral and aortic valves, cocci demonstrable; miliary abscesses and diffuse polymorphonuclear infiltration of myocardium. Superimposed infection: acute bacterial endocarditis, septicemia.

CASE 23.—White, male, aged 46 years, had pleurisy at 33 and 45 years. Frequent joint pains in hands. Admitted six weeks after onset of last illness, which began with cough and hemoptysis. Night sweats, weight loss, epistaxis, dyspnea, and anorexia. He was febrile and had many petechiae and ecchymoses. Slightly enlarged liver. No other physical signs of cardiac pathology or failure. Roentgenogram: some left ventricular enlargement and aortic dilatation. Blood Wassermann four-plus. On the fifteenth day, the temperature rose to 105° F.; on the eighteenth day, erysipelas of the nose. He died on the twenty-second day.

Pathology.—Acute active rheumatic valvulitis and myocarditis. Acute endocarditis of mitral and aortic valves. Aschoff bodies and miliary abscesses of myocardium. Miliary abscesses of spleen and kidneys with demonstrable gram-positive cocci. Superimposed infection: erysipelas.

CASE 24.—White girl, 7 years old. Rheumatic fever at 4 years. Frequent sore throats during next three years. Joint pains at intervals. Cardiac lesion known to be present. The day before admission, she was listless and developed paresis of the left leg. On admission, left hemiplegia and meningeal irritation, enlarged heart, and harsh apical systolic murmur, clubbed fingers and toes, palpable spleen, slightly elevated temperature and leucocytosis, hematuria, blood in spinal fluid. She was febrile for four weeks and died one hour after the appearance of signs of fresh intracerebral bleeding.

Pathology.—Chronic active rheumatic valvulitis and myocarditis. Acute endocarditis of mitral valve. Intense granular degeneration and polymorphonuclear infiltration of myocardium. Subdural hemorrhage from ruptured septic aneurysm of a pial artery. Acute infarctions of spleen and kidneys. Superimposed infection: septicemia of unknown origin.

CASE 25.—White, male, 19 years old, presented no rheumatic history. Admitted in first attack of polyarthritis. Typical rheumatic clinical course with recovery in two months. Readmitted seven months later, ill one week with generalized aches and pains, joint pains and fever. He had petechiae and purpura, was somnolent, and the temperature was elevated to 104.2° F. The spinal fluid contained blood. Blood culture revealed hemolytic *Staphylococcus aureus* bacteriemia. Death in thirty-six hours.

Pathology.—Chronic active rheumatic valvulitis and myocarditis. Acute endocarditis of mitral and aortic valves with demonstrable cocci. Miliary abscesses of myocardium, cocci demonstrable. Multiple cerebral hemorrhages. Multiple abscesses of kidneys, liver, spleen, and brain. Superimposed infection: staphylococcus septicopyemia, origin unknown.

CASE 26.—White woman, aged 26 years. Scarlet fever in childhood. Rheumatic fever at 5, 7, and 15 years. Four months before admission, she had severe uterine bleeding after cauterization of the cervix, followed by weakness, weight loss, night sweats, chills, fever, cough,

dyspnea, and palpitation. On admission she had a septic temperature curve, mitral stenosis, an enlarged liver and spleen, and pulmonary congestion. Electrocardiogram: right axis deviation, low voltage T-waves in Lead I. Roentgenogram: mitral stenosis. Blood cultures positive for gonococci. Embolism of popliteal artery followed by gangrene.

Pathology.—Chronic active rheumatic pancarditis. Acute endocarditis of aortic valve with demonstrable organisms. Acute abscesses of myocardium with demonstrable organisms. Septic thrombi of kidneys, spleen, and popliteal artery. Superimposed infection: gonococcemia.

CASE 27.—White woman, 34 years old, had chorea and rheumatic fever at 12 years. During the seventh pregnancy, she had dyspnea and leg edema which recurred at intervals. Two months before admission, precordial pain, and slight edema appeared. Auricular fibrillation, rate rapid, pulse deficit, congestive failure; systolic and diastolic apical murmurs; venous thrombosis of right arm; irregular temperature; multiple skin ulcers. Electrocardiogram: auricular fibrillation, premature nodal contractions. Negative blood culture.

Pathology.—Chronic active rheumatic pancarditis. Acute endocarditis of mitral valve with demonstrable gram-positive cocci. Diffuse polymorphonuclear infiltrations of myocardium. Superimposed infection: acute bacterial endocarditis.

CASE 28.—White, male, aged 52 years, had pneumonia twice in childhood. Blood Wassermann, four-plus. Joint pains in third decade. Six months before admission had cough and dyspnea on exertion; three weeks before, had joint pains. Afebrile. Erythematous and macular rash on neck, hands, and chest. Roentgenogram: enlarged heart and mitral stenosis. Blood pressure 100/70 mm. Hg. No gross congestive failure. Argyll-Robertson pupils. Irregular temperature during last six days rising to 107° F.; blood culture: hemolytic streptococcus.

Pathology.—Chronic active and healed rheumatic valvulitis (mitral and aortic) and myocarditis. Acute endocarditis of mitral valve. Many diffuse foci of myocardial degeneration with polymorphonuclear cell infiltration. Recent thrombosis of coronary artery. Superimposed infection: hemolytic streptococcus bacteriemia.

CASE 29.—White woman, aged 54 years, gave no rheumatic history. Cardiac symptoms for four years. General anasarca for one year. Auricular fibrillation. Slight nitrogen retention. Irregularly febrile.

Pathology.—Healed rheumatic valvulitis (mitral) and myocarditis. Acute endocarditis of mitral valve with demonstrable gram-positive cocci. Acute fibrinous pericarditis with same organisms. Foci of myocardial degeneration with polymorphonuclear cell infiltration, same organisms. Acute pneumonitis and pleurisy. Multiple renal infarctions, same organisms. Acute enteritis. Superimposed infection: pneumonia, and acute bacterial endocarditis.

CASE 30.—White, male, 58 years of age, had rheumatism at 47 years. Three months before admission, he had an upper respiratory infection and a resultant lung abscess. Dyspnea and weight loss for one month. Mitral stenosis and auricular fibrillation. Blood culture negative. Purpura and subconjunctival hemorrhages.

Pathology.—Healed rheumatic valvulitis and myocarditis. Acute endocarditis of mitral, aortic, and tricuspid valves and mural endocardium, gram-positive cocci demonstrable. Acute myocardial abscesses with same organisms. Miliary abscesses of kidneys. Superimposed infection: acute bacterial endocarditis.

DISCUSSION

In this group of nine cases, the superimposed infections were acute and subacute bacterial endocarditis, septicemia, erysipelas, and pneumonia. Seven of the nine cases of endocarditis had gross fresh vegetations, five with organisms; two had only fibrinous exudate with bacteria. Every case had acute nonrheumatic inflammatory lesions in the myocardium, four cases with bacteria in the

miliary abscesses (Fig. 3). Three cases had rheumatic pericarditis, one with Aschoff bodies, the others adhesive. One case (29), had fibrinous pericarditis of streptococcal origin. Six cases had abscesses or septic thrombi and infarctions in the brain, liver, kidneys, or spleen. There were two cases with meningeal or cerebral hemorrhages. The characteristic features of this group of predominant superimposed infections are acute nonrheumatic inflammatory lesions of the myocardium, consisting of diffuse or focal polymorphonuclear infiltration, acute valvular lesions, multiple abscesses, thrombosis, infarctions, and hemorrhages in remote organs, bacteria being demonstrable in one or more localities. The myocardial lesions, however, differ from the cases of the preceding group only in the presence of bacteria. Two of these cases had acute active rheumatic carditis, five had chronic active, and two had healed rheumatic lesions.

SUMMARY

A study of thirty representative cases of rheumatic heart disease discloses the unexpectedly frequent presence of superimposed infections. The outstanding feature of superimposed infection is acute myocarditis, characterized by degenerative lesions of the myocardial fibers, polymorphonuclear cell infiltration, and miliary abscess formation. Other manifestations are acute and subacute bacterial endocarditis, "indeterminate," nonrheumatic or nonbacterial endocarditis, and possibly acute pericarditis. The bacterial organisms involved may be detected in the blood stream or demonstrated in the valvular vegetations, myocardial abscesses, epicardium, or remote organs. In some cases the etiologic agent can only be inferred from other complicating factors, such as erysipelas, pneumonia, scarlet fever, etc. The myocardial lesions are similar in the proved bacterial cases to those in which the organisms are not found.

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THE PRESSOR EPISODES, DIETS, AND THE TOXICITY OF MORPHINE SULFATE*

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IT HAS been shown[1] that the minimal lethal dose of morphine obviously increases the total death rate of mice on the days of pressor episodes and that ordinary alkaline or acid water in mouse diets definitely changes their reaction toward an injected minimal lethal dose of morphine sulfate. This led us to further studies in this direction, increasing the number of experimental animals, which in this case has been 1,350 mice.

The animals, approximately of the same age and weighing from 17 to 21 gm., were fed with "Special Meal" (Old Trusty Dog Food Co.). All the mice were divided into three groups of 450 animals each. The first group had ordinary tap water, the second group had water with 2 per cent of sodium citrate, and the third group had water with $\frac{1}{30}$ per cent of hydrochloric acid. The urine of the animals on tap water showed a pH of 6.0; on water with sodium citrate, pH 8.0; and on water with hydrochloric acid, pH 5.5.

Daily, for thirty consecutive days, 15 mice of each group were injected with a minimal lethal dose of morphine sulfate, taking as such 0.25 gm. per kg. of animals, which makes 5 mg. per mouse of 20 gm. of weight. Each animal was weighed separately and given the corresponding dose. The animals were observed until their recovery or until their death. The time interval between the moment of morphine injection and death for each animal was recorded separately. The findings are presented on three graphs.

On Graph I are shown daily deaths of mice. Three upper solid lines represent the daily deaths of mice in each group, control, alkaline, and acid. The fourth line represents the total deaths of mice in all three groups. The figures on the left of all these four solid lines represent the number of dead mice; the interrupted horizontal lines through the lines designate the average daily deaths.

The lower lines give meteorologic data on the designated dates (high, mean, and low temperatures and barometric pressure). The vertical lines point to the peaks, where the total number of deaths varied considerably from the average daily deaths (20.4) or from the number of deaths on the previous day. The letters at the bottom designate the days with stable meteorologic conditions *(s)*, days with pressor episodes *(p)*, and days after the days with pressor episodes *(a)*.

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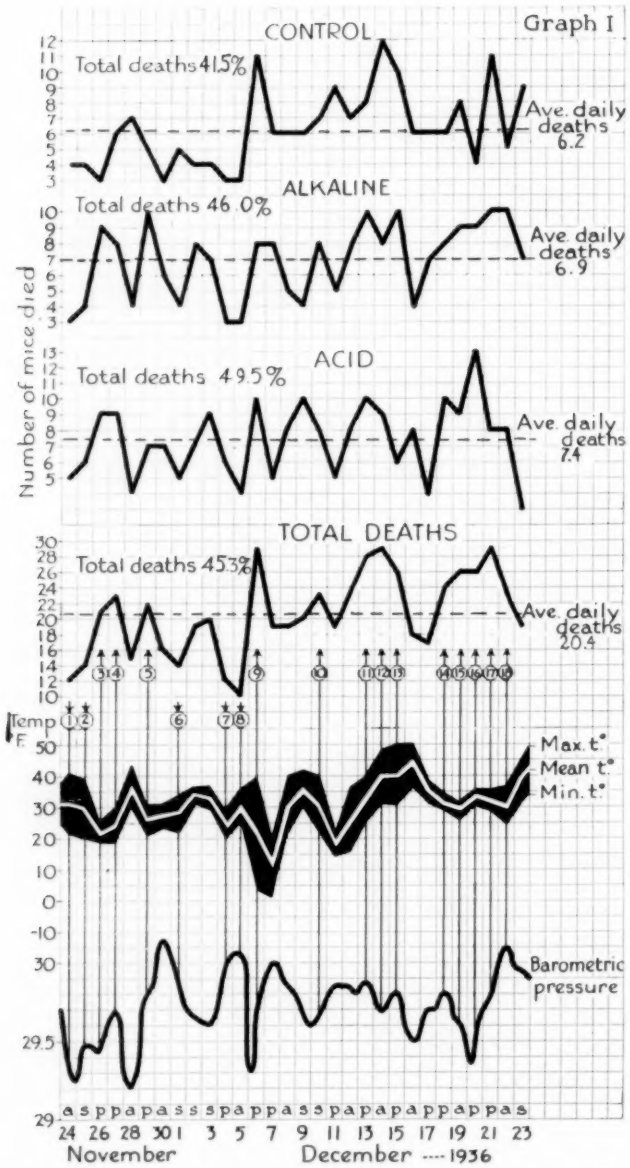

Graph I
CONTROL
Total deaths 41.5%
Ave. daily deaths 6.2
ALKALINE
Total deaths 46.0%
Ave. daily deaths 6.9
ACID
Total deaths 49.5%
Ave. daily deaths 7.4
Number of mice died
TOTAL DEATHS
Total deaths 45.3%
Ave. daily deaths 20.4
Temp F.
Max. t°
Mean t°
Min. t°
Barometric pressure
a s p p a p a s s s p a p p a s s p a p a p a p p a p p a s
24 26 28 30 1 3 5 7 9 11 13 15 17 19 21 23
November
December ---- 1936

Graph I.—Three upper solid lines represent the daily deaths of mice in each group, control, alkaline, and acid. The fourth line represents the total daily deaths of mice in all three groups. The figures on the left show the number of dead mice; the interrupted horizontal lines through the solid lines designate the average daily deaths for this particular group of mice. The wide, uneven, black line expresses the official temperature (maximum, mean, and minimum) on given days. The lower curve is a barograph, showing the official barometric pressure for the days on which the experiments were performed. The figures below give dates on which experiments were done. The letters at the bottom designate the days with stable meteorological conditions (*s*), days with pressor episodes (*p*), and days after the days with pressor episodes (*a*).

In analyzing this graph we see that the daily deaths (three upper lines) in three groups vary daily, and also vary from each other. Since each group contained daily observations only on 15 mice, it is better to analyze, first, the total number of mice, including all the three groups, 45 mice per day.

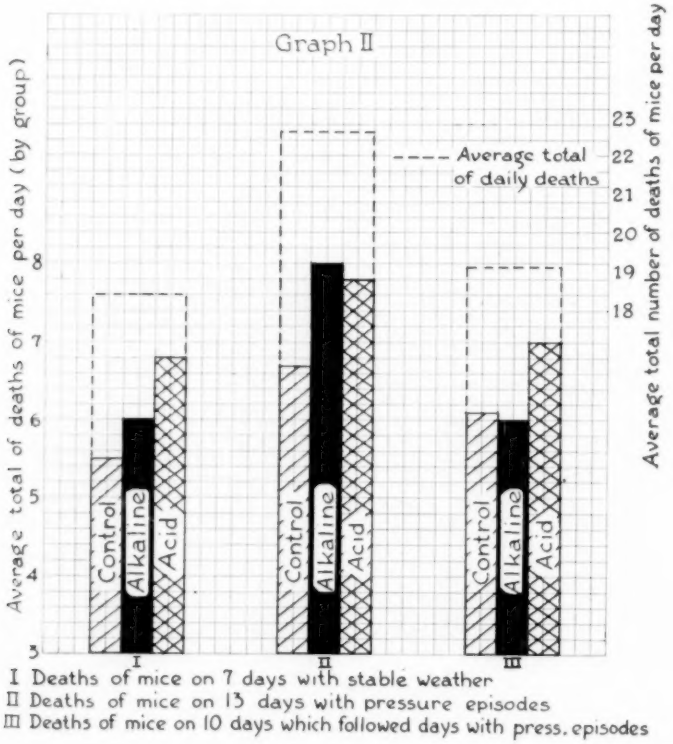
The verticals 1, 2, and 6 point to the low death rate on these days, when the temperature and the barometric pressure have been quite stable. At 7 and 8, though we observed a considerable rise in barometric pressure, the rise was relatively slow and there was little change in the temperature. Verticals 3, 4, 5, 10, and 14, pointing to the increased death rates, show on these days a fall in temperature and rise in barometric pressure. Verticals 11, 12, 13 point to days with higher death rates, when the barometric pressure is quite stable, but the temperature shows great differences between maximum and minimum. Verticals 15, 16, 17, and 18, days with low temperatures and marked changes in barometric pressure, also point to a higher death rate. The most striking observation has been recorded on the day with vertical 9. A sudden rise of barometric pressure following a sudden and deep fall is accompanied by a striking difference in maximum and minimum temperatures on that day, and the death rate, from 10 mice on the previous day rises sharply to 29 animals on the designated day.

If we also take the line between the verticals 8 and 9 and by it divide the whole chart in two parts, then we see that on the left side the death rate in general is lower and the weather is more stable (see temperatures). On the right side where the weather changes are very much pronounced, the death rate is obviously higher. The three upper lines, representing the daily deaths in three groups of mice separately, point out unquestionably again the daily variations in each group.

Since it is well established by vast clinical and experimental evidence (Petersen[2]) that the sudden changes in meteorological environment definitely change the physiologic state of an organism, we have analyzed our data presented on Graph I in the following manner. All thirty days of our observations were divided into three groups; namely, days with stable weather conditions (seven); days with pressor episodes (thirteen); and days (ten) which followed the days with pressor episodes. We summarized the total deaths in each group of days, and the total deaths in control, alkaline, and acid diet groups of animals in groups by days, and divided them by number of days in groups. This gave us average deaths by days in each group and in each diet group of the mice. The results are presented on Graph II. The figures on the left designate the average daily number of deaths by diet groups, and the figures on the right—denote the average total deaths. The three groups of columns represent: group I—the average deaths on days with stable meteorological conditions; group II—average deaths on days with pressor episodes; and group III—average deaths on days, following the days with pressor episodes. First columns in each group designate average deaths per day in control groups of animals; the central ones—the average deaths among mice on alkaline diet; and the third ones—the average deaths of mice on acid

diet. The wide columns over each group of columns, shown by broken lines, give the average total daily deaths of mice in groups of days with different meteorological conditions.

The graph shows that the total average daily deaths is only 18.4 of mice on stable days, while it is 22.6 on days with pressor episodes, and down to 19.1 on days, following the days with pressor episodes. In diet groups the control animals give average daily deaths 5.5, 6.7, and 6.1 of mice. In alkaline groups the findings are 6.0, 8.0, and 6.0. In acid groups we have 6.8, 7.8, and 7.0.

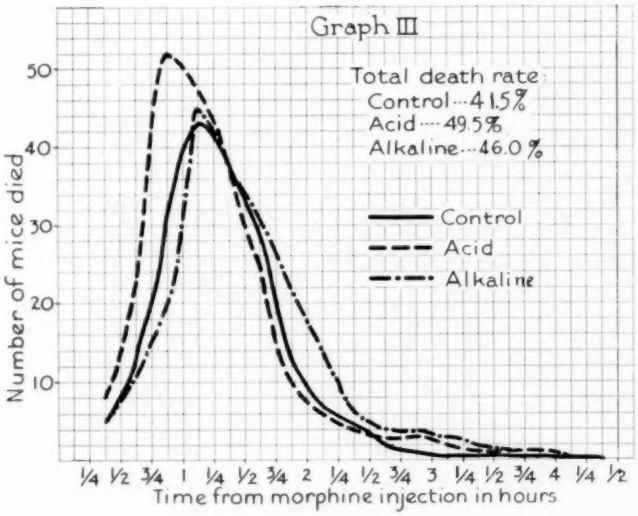

Graph II
Average total of deaths of mice per day (by group)
Average total number of deaths of mice per day
Average total of daily deaths
Control
Alkaline
Acid
I
II
III
I Deaths of mice on 7 days with stable weather
II Deaths of mice on 13 days with pressure episodes
III Deaths of mice on 10 days which followed days with press. episodes

Graph II.—The figures on the left designate the average daily number of deaths in diet groups of I, II, and III, the figures on the right, the average total daily deaths in I, II, and III. I, II, and III groups of columns represent the findings on (I) days with stable meteorological conditions, on (II) days with pressor episodes, and on (III) days following the days with pressor episodes. First column in I, II, and III designates the average daily deaths in mice on normal diet (control group); the central ones (black), the deaths among mice on alkaline diet; and the third ones, the deaths among mice on acid diet. The wide columns over each group of columns (shown by broken lines) give the average total daily deaths in I, II, and III.

The average number of deaths in alkaline diet groups is the highest on days with pressor episodes, even slightly above the deaths in group of animals in acid group, but on the days following the days with pressor episodes, the average daily deaths are lower than in acid diet group and even slightly lower than in control group. This may be explained by better buffering of the animals on alkaline diet, which facilitates their recovery after pressor episodes (Petersen[2]). The number of average deaths in acid diet groups is considerably higher than in other ones, except as mentioned above, in the

group of days with pressor episodes, where it is slightly lower than in alkaline group. Nevertheless the total number of deaths in acid mice (Graph I) is decidedly higher in comparison with the other two groups.

As mentioned previously, the time in which each mouse died, from the moment of morphine injection, has been recorded. The summary of these observations is presented on Graph III. The black curve represents the deaths of mice in control group, the interrupted line represents the deaths in acid group, and the interrupted line with dots represents the deaths in alkaline group. The figures on the left show the number of mice dead and the figures at the bottom designate the time in which the mice died from the moment of morphine injections. We can see that the mice in the acid group begin to die first and the peak of their death rate is reached in about fifty-two minutes. In about an hour later this death rate decreases approximately to



Graph III.—The black curve represents the deaths of mice in control group, the interrupted line represents the deaths in acid group, and the interrupted line with dots represents the deaths in alkaline group. The figures on the left show the number of mice dead, and the figures at the bottom designate the time in which the mice died from the moment of morphine injections.

the death rate of other two groups. Next in speed of death rate is the control group, reaching the peak in about one hour and eight minutes and beginning to decline falling later than the acid group in approximately an hour and twenty-two minutes. The death of the mice in the alkaline group, though higher than in the control group, begins later than in the control group, reaching its highest peak in an hour and seven minutes, when it starts to decline, and in an hour and twenty-two minutes it falls behind the death curve of mice in control and acid groups, remaining as such until two hours and twenty-two minutes, if not to the end of observations.

CONCLUSIONS

1. The meteorologic conditions definitely vary the death rate from a minimal lethal dose of morphine sulfate in mice on normal, acid, and alkaline diets.

2. The total death rate of animals from all three groups depends at least to a large extent on pressor episodes, which increases the death rate.

3. The mice on normal, acid, and alkaline diets give the highest average number of daily deaths on days with pressor episodes, lower on days following the days with pressor episodes, and the lowest on days with stable meteorological conditions.

4. The animals in group on acid diet die in larger number and quicker than in other two groups.

5. The death rate in control group is lower than in other two groups, and death occurs later than in group on acid diet.

6. The deaths of mice of the group on alkaline diet occur later than in both other groups. Though the total death rate is higher than in the control group, the deaths occur later than in that group.

I am greatly indebted to Dr. Bernard Fantus for his suggestions and advice.

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LABORATORY METHODS

THE COLLOIDAL CARBON FLOCCULATION TEST IN SPINAL FLUID FOR THE DIAGNOSIS OF NEUROSYPHILIS*

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THE value of the available tests done on cerebrospinal fluid for the diagnosis of neurosyphilis is well known. However, because of the wide margin of false reports that may occur,[1, 2] too great dependence upon them is not justifiable. Although this fallibility generally is recognized, the diagnosis of neurosyphilis is hesitantly made if the spinal fluid serology is negative.

To confirm the results of the accepted specific tests, another procedure employing a new method and based, possibly, on different principles would be of value. Another test whose specificity and sensitivity were such that it could give diagnostic aid where the present accepted tests failed would be of even greater value.

The use of colloidal carbon solutions as test media for spinal fluids is not new. Looney[3] demonstrated the stability of a colloidal suspension of carbon and its resemblance to Lange's colloidal gold in paretic spinal fluid. Looney and Stratton[4] described in detail its sensitivity, stability, and method of preparation by electrolysis between two carbon electrodes in a 0.033 N chromic acid bath.

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Benedek and Thurzo[5] employed a colloidal carbon solution in the elaboration of a test on spinal fluid, the Kolloid-Schutz-Index, which they believed had value in the differential diagnosis of nervous diseases. Much controversy has arisen since their work was repeated by Sacristan, Salas, and Fanjul[6] and others[7-10] on the relative value of the Kolloid-Schutz-Index.

A preliminary report on a colloidal carbon flocculation test in spinal fluid by Schube and Harms[11] appeared in 1932. The reagents employed in their test are very similar to those used by Benedek and Thurzo, the procedure differing chiefly in variations of the amount of reagents used and in their dilution. In a later report,[12] Schube stated that of 552 spinal fluids in which it had been performed, the test was positive only in cases of neurosyphilis, while in all others it was negative. He could establish no relationship between the test and the spinal fluid protein, proteose, sugar, chlorides, pH, or cell count. Deadman, Elliott, and Smith,[13] employing the colloidal carbon flocculation test as described by Schube and Harms, noted that the results in cases of neurosyphilis corresponded closely with those of the Wassermann test and other colloidal tests. They noted also that the test was not upset by (a) the presence of blood in the spinal fluid, (b) the age of the spinal fluid (up to three days) so long as it remained sterile and, (c) that the reading of the test could be delayed as long as forty-eight hours.

The colloidal carbon flocculation test as described by Schube and Harms is performed as follows: Into each of six test tubes (Wassermann tubes) place 1 c.c. of distilled water. In the first of the set is placed 1 c.c. of spinal fluid. It is thoroughly mixed, and 1 c.c. of this mixture is transferred to the second tube of the set. This process is repeated through the six tubes, the last 1 c.c. being discarded. Into each of the six tubes 0.1 c.c. of 0.1 per cent oxalic acid solution is measured. Each tube is inverted several times to mix. Then into each tube is added 0.4 c.c. of 1 per cent suspension of colloidal carbon. Each tube is again inverted several times to mix. The set is kept at room temperature and read in twelve hours, as will be described later. The 1 per cent suspension of colloidal carbon is prepared by diluting 1 c.c. of Carter's black India ink No. 358 up to 100 c.c. with distilled water. Controls are set up with each spinal fluid, consisting of 1 c.c. distilled water, 0.4 c.c. of 1 per cent colloidal carbon solution, and 0.1 c.c. of 0.1 per cent oxalic acid.

The purpose of this paper is to present an analysis of the results obtained in 478 spinal fluids in which the colloidal carbon flocculation test* was performed 484 times. The cerebrospinal fluid samples used in this study were obtained from the cerebrospinal fluid laboratory of the Boston City Hospital. A group of 26 fluids from the Boston Psychopathic Hospital were also made available for study. In almost every case, the original source of the fluid was unknown. The clinical diagnosis and associated laboratory data were obtained after the statistical results of the carbon test were completed, so that, at the time the tests were being done, their interpretation might in no way be influenced by any knowledge of the clinical features of the cases.

The method employed was a duplication of the one described by Schube and Harms. In the beginning, six test tubes were used for each test. Later, five

*Hereinafter referred to as the carbon test.

tubes and eventually four were used, for it was found that the degree of sensitivity of the test was not such that more than four tubes were practical or necessary.

To standardize the interpretation of the tests and to determine whether it might have qualitative significance, the results were read in the following way (Fig. 1): If the colloidal carbon precipitated completely, leaving an absolutely clear supernatant fluid devoid of any carbon particles, the reaction was called positive and designated as No. 4. If precipitation was nearly complete, but the supernatant fluid still showed some carbon particles in suspension, the reaction was read as negative and recorded as No. 3. With some degree of precipitation, but still marked dispersion of colloidal carbon in the supernatant fluid, the test was designated No. 2. Absence of precipitation but evidence of flocculation, characterized by a slight diminution in opacity of the solution was recorded as No. 1. If the solution remained an opaque black with no evidence of any change, it was recorded as No. 0. Only a No. 4 reaction was considered positive.

Fig. 1.—Interpretation of test. Only a No. 4 reaction is considered positive. All others are recorded as negative.

RESULTS

Of the 478 cases whose cerebrospinal fluids were studied, there were 55 from persons with clinical neurosyphilis. A Wassermann reaction was reported on forty-four of these cases and a Kahn test on the remaining eleven. A tabulation of these cases and a comparison of the results obtained with the Wassermann and Kahn tests as contrasted with the carbon test are shown in Table I.

TABLE I

SENSITIVITY OF CARBON TEST COMPARED WITH THAT OF WASSERMANN AND KAHN TESTS ON THE BASIS OF ITS ABILITY TO DETECT NEUROSYPHILIS

CLINICAL DIAGNOSIS	NO. OF CASES*	NO. WITH POS. WASS. OR KAHN	NO. WITH POS. CARBON	PER CENT POS. WASS. OR KAHN	PER CENT POS. CARBON
Tabes dorsalis	21	10	9	47.6	42.8
General paresis	10	6	8	60.0	80.0
Neurosyphilis	18	8	8	44.4	44.4
Syphilitic meningitis	2	2	2	100.0	100.0
Meningovascular syphilis	2	2	1	100.0	50.0
Juvenile dementia paralytica	2	2	2	100.0	100.0
Totals	55	30	30	54.5	54.5

*This group of cases includes those treated adequately, inadequately, and not at all.

Since doubtful Wassermann and Kahn reactions cannot be evaluated statistically,[1] they were not included in the determination of the per cent of positive Wassermann and Kahn reactions, as compared to the per cent of positive carbon reactions. It is, no doubt, purely coincidental that the percentage of positive Wassermann and Kahn tests was the same as that of the carbon tests.

TABLE II

COMPARISON BETWEEN ACCEPTED TESTS AND CARBON TEST IN UNTREATED, INADEQUATELY TREATED, AND ADEQUATELY TREATED CASES OF NEUROSYPHILIS

TREATMENT	NO. OF CASES	NO. WITH POS. WASS. OR KAHN	NO. WITH POS. CARBON	PER CENT POS. WASS. OR KAHN	PER CENT POS. CARBON
No treatment	14	14	13	100.0	92.8
Inadequate treatment	15	8	8	53.3	53.3
Adequate treatment	26	8	9	30.7	34.6
Totals	55	30	30	54.5	54.5

To more fully demonstrate how closely the accepted and the new tests agree in sensitivity, a comparison is made of the results obtained in those cases which were adequately treated, those inadequately treated, and those having received no specific treatment at all prior to the performance of the tests (Table II).

TABLE III

TREATMENT	NO. OF CASES WITH POS. WASS. OR KAHN AND POSITIVE CARBON	NO. OF CASES WITH POS. WASS. OR KAHN AND NEGATIVE CARBON	NO. OF CASES WITH NEG. WASS. OR KAHN AND POSITIVE CARBON	NO. OF CASES WITH NEG. WASS. OR KAHN AND NEGATIVE CARBON
No treatment	13	1	0	0
Inadequate treatment	5	3	3	4
Adequate treatment	3	5	6	12

On nine occasions the Wassermann or Kahn was negative and the carbon positive, and vice versa.

In Table III it is seen that on nine occasions when the Wassermann or Kahn tests were positive, the carbon test was negative, and conversely, on nine occasions when the carbon test was positive, the Wassermann or Kahn tests were negative. In the group having received no treatment, there is recorded one false negative carbon reaction. Inasmuch as they were all cases of clinical neurosyphilis, the question arises as to whether the carbon test may be based on a physicochemical mechanism different from the Wassermann or Kahn tests. This difference between the carbon and the accepted tests appears to bear no relation to the amount of treatment received.

The carbon test was performed on 416 different spinal fluids in a group of patients where a clinical diagnosis other than neurosyphilis was made (Table IV). In these fluids, the Wassermann reaction was done on 206 cases, the Kahn test being performed on the remainder. On the basis of their ability to exclude neurosyphilis, the Wassermann and Kahn tests had a specificity of 99.0 per cent, and the carbon test a specificity of 98.1 per cent. The Wassermann or Kahn tests were falsely positive four times and doubtful once, while the carbon test was falsely positive eight times. The clinical diagnoses and other spinal fluid findings in these cases are recorded in Table V.

TABLE IV

SPECIFICITY OF CARBON TEST COMPARED WITH THAT OF WASSERMANN AND KAHN TESTS ON THE BASIS OF ITS ABILITY TO EXCLUDE NEUROSYPHILIS

CASES (NOT NEUROSYPHILIS)	NO. OF CASES	NO. WITH FALSE POS. WASS. OR KAHN	NO. WITH FALSE DOUBTFUL WASS. OR KAHN	NO. WITH FALSE POS. CARBON	% FALSE POS. WASS. OR KAHN	% FALSE POS. CARBON	% NEG. WASS. OR KAHN	% NEG. CARBON
Syphilis (not C.N.S.)	63	0	0	0	0	0	100	100
Misc. Neurol Diseases	124	1	0	3	0.8	2.4	99.2	97.6
Cerebral Vasc. Accidents	79	0	0	0	0	0	100	100
Epilepsy	21	0	0	0	0	0	100	100
Brain Tumor	16	0	0	0	0	0	100	100
Bacterial Meningitis	11	2	0	0	18.1	0	81.9	100
Subdural Hematoma	11	0	0	0	0	0	100	100
Multiple Sclerosis	8	0	0	1	0	12.5	100	87.5
Migraine	6	0	0	0	0	0	100	100
Encephalitis	4	0	0	1	0	25.0	100	75
Misc. Medical Diseases	73	1	1	3	1.3	4.1	98.7	95.9
Totals	416	4	1	8	1.4	2.1	99.0	98.1

In the group with falsely positive Wassermann and Kahn reactions, Case 1 affords an example of disturbance of the hemato-encephalic barrier by bacterial meningitis. The blood serology was not determined, but a clinical diagnosis of cardiovascular syphilis was appended to that of pneumococcus meningitis. At autopsy, there was found evidence of syphilitic aortitis, but so far as could be reasonably ascertained, no gross or microscopic evidence of central nervous system syphilis. Merritt and Fremont-Smith[16] state that the falsely positive spinal fluid reactions in these cases are due to passage of the "Wassermann substance" from the blood to the spinal fluid through the inflamed vessels. Cultures of the specimen of fluid studied in Cases 1 and 2 showed no growth (they had previously been positive). Cases 2 through 5 had negative blood serology and clinically showed no evidence of neurosyphilis.

In the group with falsely positive carbon reactions, it is important to note that every spinal fluid had abnormal characteristics and that almost every case presented a distinct neurologic problem. Since the spinal fluid serology was negative, a diagnosis of neurosyphilis was not made. Failure to correlate the negative test with the clinical signs tempts the clinician to make nonspecific diagnoses, such as acute encephalitis—cause unknown, or rhythmic myoclonia. Case 6 was admitted to a medical ward where a diagnosis of syphilitic meningitis was made and the patient transferred to the neurologic service. Here a diagnosis of syphilitic meningitis was also made on the basis of the clinical picture and spinal fluid findings. When the Wassermann test was reported negative, the diagnosis was deferred and eventually became acute encephalitis of unknown etiology. Case 9 was that of a 9-year-old girl with rhythmic myoclonia, who, in addition, was mentally dull and had bilaterally an old healed chorioretinitis. Examination of the visual fields showed mild contraction of the peripheral fields with increase in size of the blind spot, more marked in the left eye. In the record

TABLE V

ADDITIONAL DATA ON CASES WHERE FALSELY POSITIVE REACTIONS WERE ENCOUNTERED

NO.	CLINICAL DIAGNOSIS	SUGAR	CHLORIDES	CELLS	PROTEIN	GOLD SOL	SPINAL FLUID SEROLOGY	CARBON	REMARKS
1	Pneumococcus meningitis	36	632	19,000 93% P	144	0112332100	Pos. K	04	Also had cardiovascular syphilis
2	Meningococcus meningitis						Pos. W	2100	
3	Hysterical psychoneurosis			0	19	0001000000	Pos. K	04	Blood serology, neg. K. No f.H. or stigmas of syphilis
4	Alcohol cirrhosis of liver			L/3	29	0111000000	? W	04	Blood Hinton neg. No clin. evidence of C. N. S. syphilis
5	General arteriosclerosis			260 R.B.C.	49	0011000000	Pos. K	04	Serology repeatedly negative
6	Acute encephalitis—? Cause	65	748	L/10	38	0012332110	Neg. W	4000	Clinical diagnosis had been syphilitic meningitis
7	Multiple sclerosis	67		L/10	36	5444321110	Neg. K	4000	An only brother died at birth. Married 14 yr., no pregnancies
8	Senile psychosis			L/35 P/5	54	010	Neg. K	4000	Abnormal W. B. C. and protein
9	Rhythmic myoclonia				18	5543210000	Neg. W	4000	Patient 9 yr. old. Has old chorioretinitis
10	Senile psychosis	71	710	L/10 P/5	116	0011211000	Neg. K	4000	Abnormal W. B. C., protein, and gold curve
11	T.B.C. arthritis				35	0011211000	Neg. K	4000	Abnormal gold curve
12	Cerebral thrombosis			R/10	46	0001100000	Neg. K	4000	Cerebral thrombosis at 34 yr. Dead at 36 of cerebral hem.
13	Nephritis with uremia	63	647	L/14	35	Thymolized	Neg. K	4000	Negro. Abnormal cell count

Key: R = erythrocyte; L = lymphocyte; P = polymorphonuclear; K = Kahn; W = Wassermann.

the question of congenital syphilis was raised, and investigation of the patient's family advised. This was not done and the diagnosis of rhythmic myoclonia remained. Case 12 was that of a male of 34 years who had a cerebral thrombosis with hemiplegia. Two years later he was dead from cerebral hemorrhage. Vascular neurosyphilis is the only type of neurosyphilis in which it is generally recognized that the spinal fluid (and often the blood) may be negative.[17] Cases 8 and 10 were both diagnosed senile psychosis in spite of the abnormally high white cell counts, protein, and abnormal gold curve in the latter. Recognizing the fact that in every case the spinal fluid was abnormal and that in many of the cases there were attendant data suspicious of syphilis, one is tempted to suspect that these so-called falsely positive carbon reactions may not all be false. On the other hand, it is wise to accept the idea that at least a few are falsely positive. The frequency with which the accepted serologic tests on spinal fluid (and blood) are negative in frank cases of neurosyphilis is known.[17] The carbon test will be most valuable to fill in these gaps if it can be proved to have the degree of sensitivity that suggests itself.

Two cases not included in the above groups deserve special consideration. Each was diagnosed essential hypertension with cerebral hemorrhage and subarachnoid hemorrhage, respectively. The spinal fluid red cell count was 625,000 per c.mm. in the first, and 125,000 per c.mm. in the second. Both had positive blood serology (Kahn). The Kahn test on the spinal fluid was positive in both, but the carbon test negative. In neither case was neurosyphilis suspected clinically before the spinal fluid serology was known, nor could it be definitely recognized afterwards. It is possible that the patients may have had latent or asymptomatic neurosyphilis. It is well known, however, that the presence of positive blood serum even in small amounts in the spinal fluid (as existed in these cases) may produce falsely positive Wassermann and Kahn reactions.[14, 15] The positive spinal fluid serology reports in the above cases are therefore unreliable.

Does the presence of blood in the spinal fluid interfere with the carbon test? The red cell count varied from 1,000 to 50,000 per c.mm. in twenty-three samples of fluid, from 50,000 to 100,000 in two fluids, from 100,000 to 200,000 in two fluids, was 500,000 in one fluid and 625,000 in another. Four fluids from patients with neurosyphilis giving a positive carbon reaction had red blood cell counts varying from 300 per c.mm. to 18,600 per c.mm. In all other instances, the reaction was negative even with red cell counts up to 625,000 per c.mm. One can conclude that the presence of blood in the spinal fluid up to 625,000 per c.mm. does not induce a false positive carbon reaction. Whether it may be responsible for a false negative reaction by virtue of the protein that it adds to the spinal fluid should be considered, for in the only false negative reaction obtained in this study, the spinal fluid protein content was 186 mg. per cent. The highest protein value associated with a positive carbon reaction in this study was 159 mg. per cent.

The time that the fluid may be allowed to stand before the test is performed is of no significance so long as it remains sterile. Fluids have been kept for five weeks in the refrigerator without affecting the positivity or negativity of the

reaction. Contaminated fluids, however, almost invariably induce a false positive reaction. In this study, four contaminated fluids were encountered. All of them showed active bacterial growth on culture, and all gave strongly positive carbon reactions. In one of these, the test when repeated on a fresh sterile specimen gave a negative reaction. It is interesting that in three cases of tuberculous meningitis in this series the reaction was repeatedly negative.

The addition of thymol to the spinal fluid (to preserve its sugar content) did not interfere with the accuracy of the test in one case with and one without neurosyphilis.

COMMENT

The addition of another diagnostic test for the detection of neurosyphilis to the many existing ones would ordinarily create no unusual interest if it did not offer advantages over the already accepted procedures. The colloidal carbon flocculation test in spinal fluid appears to have specificity equal to and sensitivity possibly greater than the Wassermann and Kahn tests. It employs reagents that are readily available, stable, and inexpensive. No special apparatus is necessary. The test can be done as easily by the physician in his office and by the average technician in the average routine laboratory, as by the skilled technician in the higher grade laboratory. The interpretation of the reaction is simple and requires no unusual skill or training. For these reasons, the carbon test deserves more extensive trial, study, and experimentation.

SUMMARY

1. In 55 cases with clinical neurosyphilis the total incidence of positive Wassermann and Kahn reactions was identical with that of positive carbon reactions.

2. Of 14 untreated cases of neurosyphilis, with one exception, the carbon reaction matched the positivity of the Wassermann and Kahn reactions.

3. Of 15 inadequately treated cases of neurosyphilis the Wassermann or Kahn tests were positive eight times, and the carbon test was positive eight times.

4. Of 26 adequately treated cases of neurosyphilis the Wassermann or Kahn tests were positive eight times, while the carbon test was positive nine times.

5. Of 416 cases without neurosyphilis a false positive Wassermann or Kahn reaction was encountered four times, while a false positive carbon reaction was encountered eight times.

6. There is reason to infer that some of the "falsely" positive carbon reactions were positive.

7. The integrity of the carbon test is not altered by (a) blood in the spinal fluid (up to 18,500 red cells per c.mm.), (b) standing in a refrigerator for five weeks, and (c) thymolyzing the fluid.

8. Contamination of the spinal fluid by actively growing bacteria produces falsely positive carbon reactions.

9. The carbon test is positive only when there is complete precipitation of the colloidal carbon leaving an absolutely clear supernatant fluid devoid of any carbon particles. Any other reaction is to be considered negative.

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A PERFUSION STAGE FOR OBSERVATIONS ON DAPHNIA*

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IN THE course of routine examination of *Daphnia magna*, the need of a suitable apparatus for confining the animal to the field of vision and facilitating rapid and accurate change of media became apparent. This led to the development of the perfusion stage described below which has proved quite satisfactory. With slight modifications in the construction of the stage itself, the apparatus should lend itself to observations on related crustaceans, small aquatic worms, larval forms, etc.

The complete apparatus (Fig. 1) consists of the following parts: A constant temperature water-bath (*A*) is provided with a water inlet and overflow permitting water of the required temperature to be circulated through it. Two culture medium containers (*B, C*) are held in place in the chamber by the large two-hole stopper at the bottom of the constant temperature bath. One container

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is filled with the modified medium to be studied, and the other with the control medium. The flow of either of these media is controlled by the two-way stopcock (*D*). The lower, single tube of the cock is drawn to a point and connected to the inlet of the perfusion stage by a piece of narrow rubber catheter. The taper of this lower tube should begin immediately below the cock itself to minimize, as far as possible, leftover solution from one medium mixing with the second medium. The change of media should be as abrupt and complete as possible.

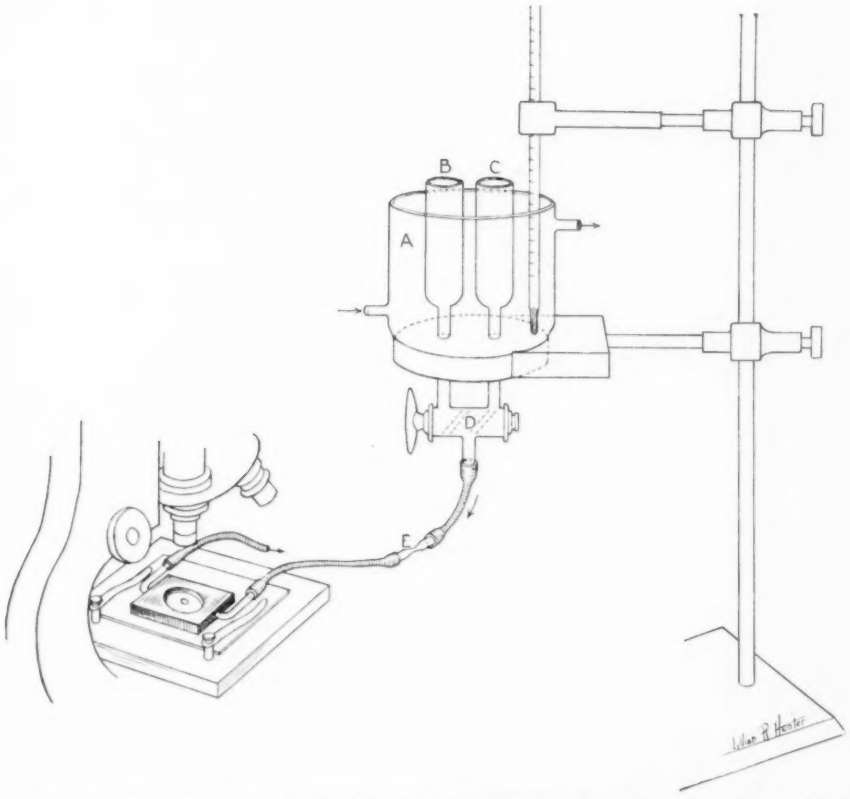

Fig. 1.—Perfusion stage and temperature controlling bath. *A*, Water-bath provided with inlet and outlet; *B*, *C*, culture medium containers; *D*, two-way stopcock for controlling culture medium flow; *E*, constriction tube to reduce rate of flow.

It has been found advisable to insert a constricted capillary tube in the inlet connection (*E*) to maintain a steady even flow through the chamber when the cock is wide open. The perfusion stage outlet is composed of a second length of fine catheter, allowing the waste solution to escape into a beaker. The tubing should be as short as possible as the weight of the column of water in a longer tube is often sufficient to draw air in under the cover glass on the stage.

The construction of the stage itself is shown in Fig. 2. This is simply a section of hard glass tubing, the upper and lower faces of which are finely ground, with two capillary tubes sealed into it for inlet and outlet of the medium. The all-glass construction permits complete visibility and renders the problem of cleaning simple.

The best results are obtained with a capillary or dissecting microscope, as its very low and intermediate magnifications are best suited to observations on the daphnia.

In using this apparatus the following procedure has been found satisfactory. The condenser is racked up close to the stage and the diaphragm opened. The stopcock is closed, and the media are placed in the containers and allowed to reach the temperature desired.

A cover glass whose diameter is the same as the outside diameter of the stage is secured to the upper surface of the stage with a thin film of petrolatum. The stage is then inverted so as to rest on the cover slip. The lower surface of the stage is likewise covered with a film of petrolatum and the flow of culture medium started by opening the stopcock. As soon as all the air is out of the apparatus the stopcock is closed, leaving a pronounced meniscus at the surface of the stage. A small spot of petrolatum is placed in the center of a microscope slide. The daphnia to be observed is picked up in a

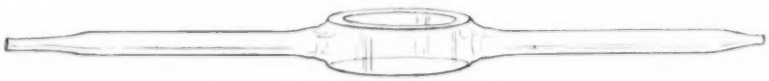

Fig. 2.—Perfusion stage (actual size).

pipette and dropped onto a towel. The slide is then inverted over the daphnia, and the spot of petrolatum brought gently but firmly into contact with the side or back of the animal. A very slight pressure will fix daphnia in place without injury. The slide and affixed daphnia are then picked up and the daphnia brought into contact with the meniscus. The microscope slide is allowed to settle in place, the excess medium escaping until the slide is in contact with the petrolatum film. The slide is pressed into the film which is sufficient to hold the stage in place. The slide and stage are then turned right side up and can be placed on the microscope stage.

The stopcock is then opened gradually until the waste fluid is dropping from the outflow at the rate of one or two drops per second. The capillary microscope is swung into place, and observations can be made.

By means of the above apparatus, the daphnia is held without harm or interference fixedly in the observation field and is bathed in a constantly perfusing nutritive medium, which also protects the animalcule against the heat of the microscope lamp. The solutions can be changed rapidly at will, and, by shutting off the flow for periods, conditions of asphyxia are easily produced.

MODIFICATION OF THE TITAN YELLOW METHOD FOR THE DETERMINATION OF SMALL AMOUNTS OF MAGNESIUM IN BIOLOGICAL FLUIDS*

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IN 1934 a simple method was published[1] for the colorimetric determination of small quantities of magnesium in biological fluids. The method was based on Kolthoff's observation[2] that magnesium imparts a red color to alkaline solutions of titan yellow. The red dye lake thus produced was shown to be directly proportional to the concentration of the magnesium present.

With this method it is necessary to add an excess of the titan yellow. A definite percentage of the dye "combines" with or "absorbs" the magnesium ion to produce the characteristic red magnesium dye lake. The surplus yellow dye remaining in the solution vitiates the red color to be compared. Consequently, unless the concentration of magnesium in the two solutions to be compared (standard and unknown) be within close range, satisfactory color matches cannot be obtained. Due to these difficulties a modification in the method which would eliminate these objections is desirable.

It has been found that the percentage of dye which "combines" with the magnesium is directly proportional to the concentration of magnesium present in the solution. Conversely then, the percentage of dye in excess to that which "combines" with the magnesium is inversely proportional to the concentration of the magnesium in the solution (see Fig. 1). Therefore, the concentration of the surplus dye remaining in an unknown solution, when compared with the surplus dye remaining in a standard solution, should be an accurate index as to the amount of magnesium present in the unknown. This, in brief, is the principle upon which is based the following method for the determination of this ion which, we believe, eliminates the objections of the older method.

METHOD

In the method reported in this paper the determination of magnesium depends upon a comparison between the intensity of the excess dye remaining in a standard and an unknown solution. An equal quantity of the same solution of titan yellow is added to the known and unknown solutions. The red magnesium dye lake is centrifuged out, and comparisons made with the supernatant fluids. Since yellow is a difficult color to match colorimetrically, an excess of magnesium is added to an aliquot part of the supernatant fluid. The magnesium thus converts the titan yellow quantitatively to the pure red magnesium dye complex. The red then can be satisfactorily matched in the colorimeter.

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In Fig. 1 is shown the inverse relationship existing between the excess dye and concentration of magnesium. In this figure the concentration of excess dye in per cent (of the total amount originally added) is plotted along the ordinates. The concentration of magnesium, in milligrams, is plotted along the abscissa. The points, except at the two extremes, fall very nearly on a straight line.

It must be pointed out, however, that titan yellow evidently is not a pure dye. Curves *A* and *B* in Fig. 1 show the results of two freshly prepared samples of the dye of equal concentrations. Curve *C* in the same figure shows the results obtained with the same solution of dye as that used in curve *B* after standing for ten days. Although the actual percentage of excess dye remaining in the solutions may vary, the straight line relationship, in each case, is maintained. The

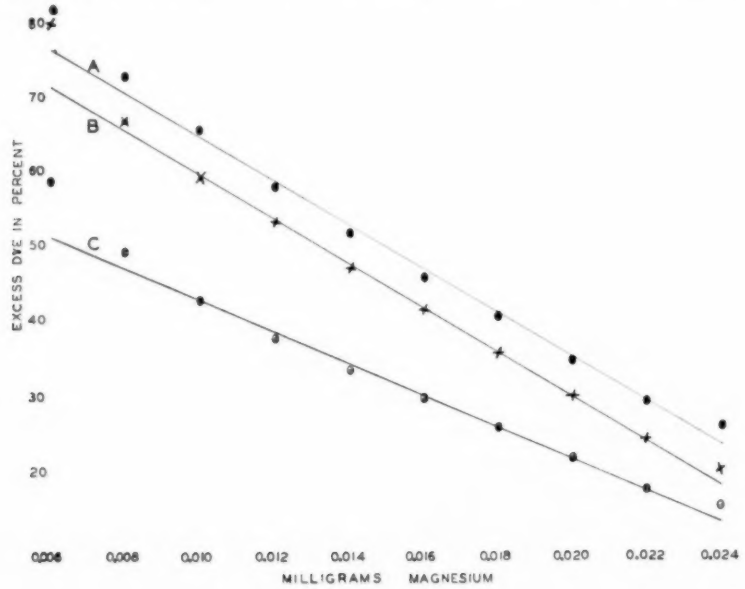

Fig. 1.

actual purity or concentration of the dye, therefore, is of no great importance. It is important, however, that the same solution of dye be used in any one set of determinations.

In making calculations for the concentration of the ion in the unknown the inverse ratio of the colorimetric reading must be employed rather than the direct ratio as is commonly employed. That is, the greater the dye concentration remaining in the supernatant fluid of the unknown, the less will be the concentration of the magnesium.

The general formula for the computation of milligrams per 100 c.c. of the unknown is:

$$\frac{\text{Reading of the unknown} \times C \times 100}{\text{Reading of standard (20)} \times V} = \text{mg. of Mg per 100 c.c. of unknown}$$

C = concentration of magnesium in the standard

V = volume of the unknown used.

In Table I is shown the results of analyses of a series of watery solutions containing known amounts of magnesium. The standard used was 1 c.c. of the working standard *b* (see solutions), i.e., C = 0.02. Results within 5 per cent error were obtained if the unknown contained no less than 0.012 or more than 0.022 mg. magnesium.

TABLE I

Analysis of a Series of Solutions of Known Magnesium Content with the Titan Yellow Colorimetric Method. The Standard in Each Case Contained 0.02 mg. of Mg (i.e. 2.0 mg. per 100 c.c.).

MG. OF Mg IN KNOWN SOLUTION	MILLIGRAMS FOUND	ERROR IN PER CENT
0.006	0.0073	21.7
0.008	0.0092	15.0
0.010	0.0108	8.0
0.012	0.0127	5.8
0.014	0.0146	4.3
0.016	0.0164	2.5
0.018	0.0183	1.6
0.020	0.0201	0.5
0.022	0.0225	2.3
0.024	0.0271	15.0

Determination of Magnesium in Serum.—The serum proteins, which act as protective colloids, must be removed before the magnesium dye complex can be centrifuged out. This is best done with trichloracetic acid. This has an advantage in that the protein-free filtrate is water clear. The color of whole serum ranges from a normal yellow to a frank red if hemolysis has taken place. This color, unless removed, would introduce a considerable error.

Calcium, as in the original method, must be removed. As ammonium oxalate was found to introduce some error, this is done with sodium oxalate.

Solutions.—All the solutions should be made with magnesium-free distilled water. The water may be tested for magnesium by adding 1 c.c. of the dye solution to 7 c.c. of the water and 2 c.c. 4 N NaOH. If no red precipitate appears upon centrifuging, magnesium is absent.

1. *Standard Magnesium Solutions.*—(a) Stock standard: weigh exactly 10.131 gm. of dry reagent $MgSO_4.7H_2O$ and dissolve in distilled water. Add 0.5 c.c. chloroform and make up to 1,000 c.c. in a volumetric flask (1 c.c. = 1.0 mg. Mg). This solution will keep indefinitely if stored in a dark place.

(b) Working standard: dilute 2.0 c.c. of the stock standard to 100 c.c. (1 c.c. = 0.02 mg. Mg).

2. Titan yellow* solution, 0.05 per cent. This solution will keep for one to two weeks if kept out of the light and in a pyrex container.

3. Trichloracetic acid (Mercks), 10 per cent. Will keep in an icebox for three to five days.

4. Sodium oxalate, reagent, 0.5 per cent.

*Titan yellow can be obtained from Arthur H. Thomas Co., Philadelphia.

5. Starch solution, 1.5 per cent. Dissolve by boiling 1.5 gm. soluble starch (Mercks) in 15 c.c. water and dilute up to 100 c.c. The solution may have a slight turbidity which will not introduce an error as equal quantities are added to all samples to be determined. This solution should be made fresh every day.

6. Sodium hydroxide, 16 per cent (approximately 4 N). A C.P. or reagent grade of sodium hydroxide should be used. A precipitate will appear in a few days which should not be disturbed. The clear supernatant solution may be siphoned off.

7. Magnesium sulfate solution, 0.3 per cent. Dissolve 0.3 gm. $MgSO_4.7H_2O$ in 100 c.c. water.

Procedure.—To 3 c.c. serum add 3 c.c. distilled water and 3 c.c. 0.5 per cent sodium oxalate. Mix and let stand at 45° C. for one hour or overnight. Centrifuge at 1,800 revolutions per minute for ten minutes. Transfer 6 c.c. of the supernatant liquid (2 c.c. serum) to a test tube and add 4 c.c. of 10 per cent trichloracetic acid. Stopper and shake vigorously. Let stand, with shaking at intervals, for one-half hour. Centrifuge for a few minutes and filter through an ash-free filter paper. Five cubic centimeters of the clear protein-free filtrate is equivalent to 1 c.c. serum. Transfer 4 c.c. of the filtrate* (0.8 c.c. serum) to another centrifuge tube and add exactly 1.0 c.c. of the dye solution. Add enough distilled water (3.0 c.c.) to bring the total volume up to 8.0 c.c. Finally, add 2.0 c.c. of the sodium hydroxide and mix. Let the mixture stand for fifteen to thirty minutes, and centrifuge at 1,800 revolutions per minute for ten minutes.

The standard is to be prepared simultaneously as follows: To 1 c.c. of the working standard (b) (0.02 mg. Mg), add 6.0 c.c. distilled water, 1 c.c. of the dye solution, and finally 2.0 c.c. sodium hydroxide. Mix and centrifuge. After centrifugation, the magnesium dye complex will appear as a red precipitate. The yellow supernatant solution will contain the excess dye. Five cubic centimeter aliquots of the supernatant liquids are carefully pipetted from the unknown and standard specimens and transferred to another set of test tubes properly labeled. To each aliquot part, add 3.0 c.c. starch solution, 1 c.c. sodium hydroxide, and finally 1 c.c. of the 0.3 per cent magnesium sulfate solution. Stopper and mix immediately.

Compare the unknown with the standard set at 20 mm. A blue glass filter in the eyepiece of the colorimeter will greatly facilitate the comparisons.

Calculation:

$$\frac{\text{Reading of unknown} \times 0.02 \times 100}{\text{Reading of standard (20)} \times 0.8} = \text{mg. Mg per 100 c.c. serum}$$

Reduced to simple terms this formula becomes:

Reading of unknown $\times$ 0.125 = mg. Mg per 100 c.c. serum.

*If the serum magnesium is above 3.0 mg. per 100 c.c., smaller aliquots of protein-free filtrate should be used. Best results are obtained if the magnesium content of the unknown is kept below that of the standard. For the great majority of cases 4 c.c. filtrate (equivalent to 0.8 c.c. serum) is the ideal quantity to use. If the magnesium content of the unknown is low, larger aliquots should be taken.

The results of serum analysis from the blood of various laboratory animals are shown in Table II.

TABLE II

Titan Yellow Colorimetric Determination of Magnesium in the Blood Sera of a Number of Experimental Animals. The Results Are Recorded in mg. per 100 c.c. of Blood Serum.

SPECIES	NUMBER OF ANIMALS	QUANTITY OF MAGNESIUM IN MG. PER 100 C.C. SERUM		
		LOWEST VALUE	HIGHEST VALUE	AVERAGE FOR SERIES
Guinea pig	24	1.83	2.16	1.91
Cat	6	1.92	2.49	2.18
Monkey	2	2.19	2.30	2.25
Dog	17	1.83	3.10	2.42
Rabbit	10	1.90	3.59	2.78

The results on 3 samples of dog serum to which known increments of magnesium have been added are shown in Table III.

TABLE III

A table showing the reliability of the titan yellow method and revealing the fact that the precipitation of the blood proteins with trichloracetic acid does not carry down any of the magnesium added to the blood serum. In this investigation the sera of known magnesium content of 3 normal dogs were used. To a given quantity of serum was added a calculated amount of magnesium before the precipitation of the proteins. Determinations of the magnesium content were then made on this modified blood serum. These results were then compared with that of the normal to determine if any of the magnesium added to the blood serum was lost.

MG. MAGNESIUM PER 100 C.C. OF CONTROL BLOOD SERUM	MG. MAGNESIUM ADDED PER 100 C.C. OF CONTROL SERUM	MG. MAGNESIUM FOUND IN EACH 100 C.C.	ERROR IN PER CENT
1.95	0.2	2.17	0.9
1.95	0.5	2.50	2.0
1.95	0.7	2.59	2.2
1.95	1.0	3.04	3.0
1.95	1.0	3.02	2.4
1.95	2.0	3.90	1.3
2.43	0.5	2.87	2.0
2.43	1.0	3.50	3.3
2.16	0.3	2.45	0.4
2.16	0.8	3.02	2.0

Magnesium Determinations in Urine.—The magnesium content per 100 c.c. urine varies greatly. For the majority of determinations 0.2 c.c. is the ideal quantity to use. It is advisable to run urines in duplicates, using 0.1 c.c. and 0.2 c.c. samples respectively. The sample matching the standard most closely should be used for the determination. With a little experience one will be able to tell at a glance if the quantity chosen contains too much or too little magnesium. As with serum most accurate results are obtained if the magnesium content of the unknown is kept between 0.012 and 0.022 mg.

Trichloracetic acid need not be used unless the specimens contain albumin or are highly colored with blood, bilirubin, etc. In such cases an equal volume of 5 per cent trichloracetic acid is used. The calcium in every specimen must first be removed. The phosphates and other constituents of the urine do not interfere. If the urines are highly alkaline and show precipitation of phosphates, they should be made neutral to litmus with concentrated HCl and filtered.

Procedure.—To 2 c.c. urine, add 8 c.c. of 0.5 per cent sodium oxalate. Let it stand for one hour at 45° C. and then centrifuge. Transfer 1 c.c. of the supernatant fluid (0.2 c.c. urine) to another centrifuge tube, add 1 c.c. of the dye solution, 6 c.c. distilled water, and finally 2 c.c. of 4 N NaOH. Mix and centrifuge. The procedure from here on is exactly as described for serum. The calculation becomes, if 0.2 c.c. urine is used:

$$\frac{\text{R. U.} \times 0.02 \times 100}{\text{R. S. (20)} \quad 0.2} = \frac{\text{R. U.}}{2} = \text{mg. Mg per 100 c.c. urine}$$

TABLE IV

Showing the Results of the Colorimetric Determination of Magnesium in the Urine of 5 Human Subjects. The Results Are Compared With the Gravimetric Determination of McCrudden.

SUBJECT	AMOUNT OF MG. FOUND (PER 100 C.C. URINE)		DIFFERENCE IN PER CENT
	Colorimetric	*Gravimetric*	
1	10.7	11.3	5.6
2	17.2	15.9	7.5
3	11.7	11.4	2.5
4	9.1	8.8	3.3
5	12.6	12.2	3.2

In Table IV is presented the magnesium content per 100 c.c. urine of 5 normal individuals, compared with the results obtained with the gravimetric method of McCrudden.[3]

SUMMARY

1. A revised method is presented for the titan yellow colorimetric determination of small amounts of magnesium in water, blood, and urine.

2. Results are given for the magnesium content of the sera of guinea pigs, monkeys, dogs, cats, and rabbits.

3. Results of urinalysis for magnesium by the titan yellow method are compared with the results obtained with the gravimetric method of McCrudden.

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A RESTRAINING DEVICE FOR UNANESTHETIZED MONKEYS*

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DURING a recent investigation† of the influence of lesions of the cerebral cortex upon surface temperature in monkeys, it was necessary to make frequent determinations of temperature over long periods in the unanesthetized animal by means of thermocouples attached to the palmar and plantar surfaces. The ordinary devices for restraining laboratory animals usually cause considerable obstruction of the circulation to the extremities. This is especially true in monkeys. Since any significant obstruction of this nature would have invalidated our temperature readings, an attempt was made to construct a restraining device which would prevent gross movement without causing undue excitement in the animals and without interfering significantly with the circulation to the arms and legs.

The restraining device, or "holding box," constructed for the purpose mentioned above, is relatively simple and has proved very satisfactory for both spider and sooty mangabey monkeys. It is essentially an oblong boxlike affair made of wood, with adjustable false back (Figs. 1 and 2, *A*) and sides (Fig. 2, *D*). The neck holder (Figs. 1, 2, and 3, *B*) and arm rest (Figs. 1, 2, and 3, *C*) are also adjustable. Attached to the undersurface of the arm rest are two pieces of sheet metal (Fig. 3 *C*, *b*) which serve as supports.

The essential dimensions of the box and adjustable parts are shown in Figs. 1 and 2. Because of the adjustable features it is possible to employ the device for monkeys of varying sizes. It has been found suitable for animals ranging in weight from 3 to 7 kilograms.

It will be noted that the floor of the box extends outward for several inches to serve as a leg rest. Medium-sized screws for securing thongs are arranged on both the arm and leg supports. The thongs are placed across the arms and legs instead of being tied around these extremities as is frequently the case in restraining laboratory animals.

In spite of their remarkable agility, monkeys properly placed in this type of holding box are unable to free their arms and legs because the adjustable neck holder, false back, and sides permit graded reduction of the internal diameter of the box to the extent necessary to prevent gross trunk movements.

At the base of the box, in the rear, is an oval-shaped opening through which the tail of the monkey may be extended. This makes for comfort of the animals,

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especially those with large tails, and, at the same time, facilitates holding the animal in the box while the necessary adjustments of the neck holder, etc., are in progress.

After the animal is placed in the box and its tail threaded through the opening in the rear, the false back is slipped into position, the arm rest with the attached chest support (see Fig. 3 *C*, *a*) is put into place and adjusted, and then the neck holder is inserted and adjusted. We have kept unanesthetized monkeys restrained in this fashion for periods, ranging from one to four hours, with little

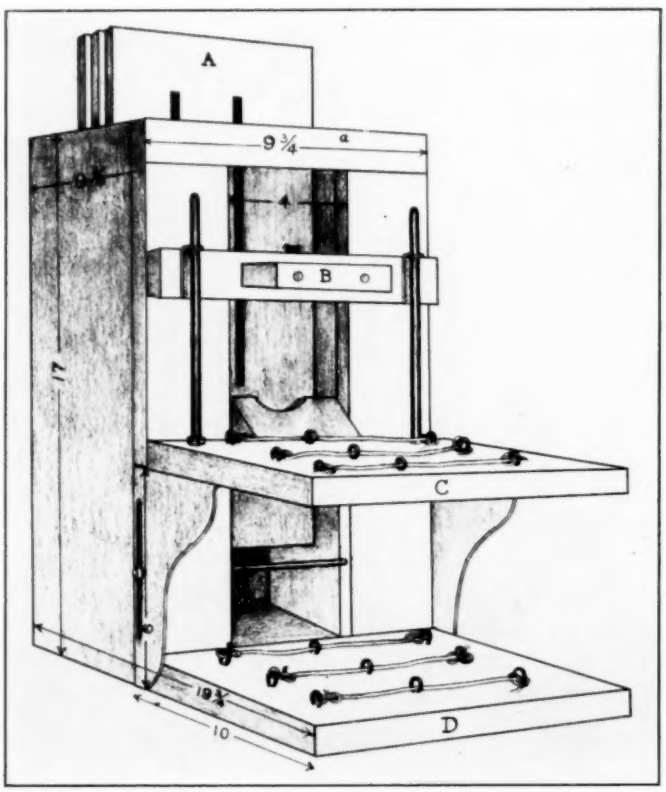


Fig. 1.—Front view showing the position of the neck holder, *B*; arm rest, *C*; and false back, *A* (partially withdrawn). The dimensions are in inches.

apparent discomfort of the animal. After a few experiences with the box the majority of the animals appeared perfectly contented while being restrained therein, and took food and water as readily as they would have if free in large cages.

Although this holding box has proved exceedingly satisfactory for our work, there is one modification which would, in the opinion of the author, facilitate placing the animal in the box. Instead of being permanently fastened in place, the cross support labelled "a" in Fig. 1 should be hinged at one end so that it can be moved out of position when desirable.

Our own experience with this type of holding box has been limited to a study of surface temperature in unanesthetized monkeys, but it might also be used for restraining monkeys during the recording of electrical potentials from

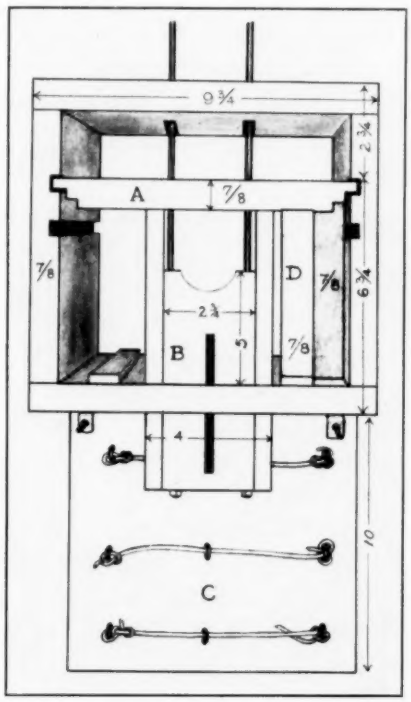
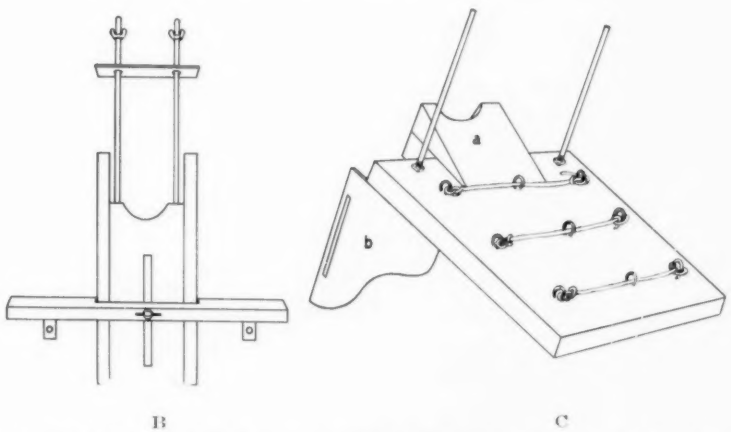


Fig. 2.—End view to illustrate the arrangement of the false sides, *D*. Note that only one of these is shown in position. The dimensions are in inches.



B C

Fig. 3.—Line drawings to illustrate the essential structural features of the adjustable neck holder, *B*, and of the arm rest, *C*, with the attached chest support, *C*, *a*.

the brain, during the photographic recording of eye movements or pupillary changes, during the determination of blood pressure by the cuff method, or during intraperitoneal or intravenous injections.

LIMITS OF USEFULNESS OF THE DIRECT AND INDIRECT METHODS OF DETERMINING VENOUS PRESSURE IN MAN*

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DURING the development of a method for measuring the venous pressure at the ankle while walking[2] the use of both the direct and indirect methods was considered. The limits of usefulness of the two methods had not been clearly set out in the literature, and it was thought worth while to make the following comparative study of the advantages and limitations of the two.

In the literature on the subject of venous pressure many partisans for each method can be found. However, there are no articles which present over an adequate range careful simultaneous comparisons of the two methods and which set out the advantages and limitations of each.

For example, accounts of comparative data which have been published are usually limited to brief statements such as those of Carrier and Rehberg,[3] who say that the indirect capsule method was checked with the direct ". . . in a few cases. . . . The agreement was excellent"; or as that of Hooker and Eyster,[6] who compared the two methods on the *exposed* vein of a dog. As might be expected, in this case close agreement was found, though only 4 values, all below 14 cm. water pressure, were presented. Again, Villaret[9] presents a few values from 6 to 15 cm. water on patients tested by both methods and with a short time interval intervening. Here the agreement between the two is occasionally very poor. In our study the two methods have been carefully compared by simultaneous readings over a wide range (5 to 121 cm. water) under clinical conditions, and the boundaries of the field of usefulness of each marked out.

METHODS EMPLOYED

The methods used will not be described in detail here, since careful description of the technique of each can readily be found in many places.

The direct method of determining venous pressure was developed by Moritz and von Tabora.[7] *This depends upon the cannulation of a vein and the free flow of normal saline into it until the pressure in the vein counterbalances the column of fluid in the manometer.* (See Eyster for references to articles which describe in detail the technique of the method.) In the present study pain was minimized with a procaine wheal before a No. 18, 1½, sharp cannula (Vim) was inserted into the vein. This was connected with a manometer filled with normal

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saline. Facilities for washing out the apparatus were included in the system, as described by Moritz and von Tabora. With this method it is important that the trauma be as little as possible, and that hematomas be avoided. If a hematoma develop, even of small extent, the experiment must be discarded. It is essential to wait until the level of saline in the manometer has come to rest. This end point should be repeatedly checked. This may be done by refill of the manometer or by brief obstruction of the vein above the cannula. All motion of the subject as well as changes in the character of breathing must be avoided until the end-point level is reached. The experiment must be abandoned when the flow of saline into the vein becomes slowed.

The principle of the indirect method of determining venous pressure as used nowadays was first worked out by von Basch.[1] *Air is introduced into a transparent capsule which is sealed over a vein until the vein collapses. The pressure at which collapse occurs is read on a manometer and represents within the limit of experimental error the pressure in the vein.* For references to the various modifications of practice in the application of this method, as well as a discussion of it, see Beecher, Field and Krogh.[2] Briefly, the procedure used here involved the use of a round celluloid capsule 1.7 cm. wide and 0.7 cm. deep. Notches were filed in each side to fit accurately the vein to be observed. The capsule was securely attached to the skin by means of celloidin and then connected to a water manometer with a mercury air pump in the line. One observer watched the vein, a second observer elevated the air pressure in the system. When the vein collapsed, the stream of blood in it was divided and the manometric pressure read. This represents the venous pressure as determined by the indirect method. It is necessary with this method to use superficial veins such as those of the hand or foot and ankle, and it is important that the vein chosen have a firm, preferably a bony background. The system must be airtight.

Since one of us had had considerable experience[2] with the indirect method and the other[4] with the direct method, we decided to compare the two simultaneously.

Veins of two regions were studied: the dorsum of the hand, and the ankle. The celluloid capsule for use in the indirect method was sealed over the vein chosen for study. The tip of the cannula employed in the direct method was inserted into the same vein so that its tip lay approximately 1 cm. from the capsule. The cannula was small in relation to the diameter of the vein chosen. By actual measurement, the presence of the cannula did not affect the venous pressure in the vein. The order of employing the two methods was varied. At least two determinations were made at each level. The pressure in the vein was altered by varying the relationship of the extremity to the heart level. Since the indirect method involves a considerable "subjective" element in determining the end point, the values obtained by use of it were determined independently of values found by the direct method. No definite adjustment of the apparatus with the patient's heart level was made, as only data *comparing* the two methods were sought.

THE DATA

These data were obtained from 13 subjects. They are recorded in Table I.

TABLE I

SUBJECT	INDIRECT METHOD CM. H_2O	DIRECT METHOD CM. H_2O	PERCENTAGE VARIATION OF METHODS (DIRECT TO INDIRECT)
McG.	5.0	10.8	+116
H.B.	5.1	10.1	+ 98
H.B.	5.1	10.9	+114
H.B.	5.1	10.9	+114
B.W.	5.5	5.2	− 5
H.B.	7.0	13.1	+ 87
H.B.	7.1	14.6	+106
H.B.	7.1	13.2	+ 86
B.W.	7.5	7.0	− 7
H.B.	8.1	11.0	+ 36
B.W.	8.5	7.0	− 18
McG.	9.0	10.5	+ 17
McG.	9.0	12.4	+ 38
H.B.	9.1	9.5	+ 4
McG.	11.0	12.9	+ 17
McG.	11.0	14.8	+ 35
M.C.	12.4	12.7	+ 2
McG.	13.0	14.3	+ 10
McG.	13.0	14.3	+ 10
M.C.	13.4	12.3	− 8
M.C.	14.4	12.7	− 5
H.B.	14.6	18.8	+ 29
M.C.	15.5	13.8	− 11
M.C.	15.5	14.7	− 5
H.B.	15.6	17.0	+ 9
McG.	17.0	17.1	+ 1
McG.	19.0	16.7	− 12
McG.	19.0	17.1	− 10
W.R.	20.4	20.9	+ 3
W.R.	20.5	20.2	− 1
W.R.	21.1	21.0	0
W.R.	21.6	21.0	− 3
B.W.	21.9	25.5	+ 16
B.W.	22.9	24.9	+ 9
H.B.	23.8	26.8	+ 13
D.F.	25.5	26.7	+ 5
M.C.	26.0	25.4	− 2
M.C.	26.0	25.4	− 2
M.C.	26.0	26.0	0
D.F.	26.5	28.0	+ 6
Se.	27.7	26.1	− 6
Se.	27.7	26.1	− 6
Bo.	29.0	26.9	− 7
Bo.	29.0	27.1	− 7
D.F.	31.5	29.8	− 5
B.W.	34.3	38.5	+ 12
B.W.	35.4	38.6	+ 9
J.C.	37.0	34.2	− 8
J.C.	37.0	34.3	− 7
J.C.	37.0	34.3	− 7
J.C.	37.0	34.3	− 7
Se.	38.0	32.2	− 15
Se.	38.0	32.4	− 15
McD.	41.1	42.8	+ 4
McD.	41.1	42.8	+ 4
McD.	43.2	42.2	− 2
McD	43.2	42.2	− 2
Se.	44.1	38.8	− 12
McD.	53.9	53.6	− 1
McD.	55.9	53.0	− 5
McD.	55.9	53.4	− 4
McD.	59.9	61.7	+ 3

TABLE I—CONT'D.

SUBJECT	INDIRECT METHOD CM. H_2O	DIRECT METHOD CM. H_2O	PERCENTAGE VARIATION OF METHODS (DIRECT TO INDIRECT)
McD.	62.0	61.4	− 1
McD.	64.0	61.1	− 5
E.F.	64.0	75.0	+ 17
E.F.	64.0	76.0	+ 19
E.F.	65.5	74.0	+ 13
E.F.	65.5	75.5	+ 15
McD.	66.0	61.3	− 7
E.F.	71.5	84.0	+ 17
E.F.	75.5	84.2	+ 12
A.H.	86.5	80.5	− 7
A.H.	90.5	82.5	− 9
A.G.	114.0	116.5	+ 2
J.C.	115.0	117.1	+ 2
J.C.	115.0	117.3	+ 2
A.G.	116.0	115.0	− 1
A.G.	116.0	115.0	− 1
J.C.	117.5	117.0	0
J.C.	117.5	117.1	0
A.G.	118.0	116.0	− 2
A.G.	118.0	116.5	− 1
J.C.	119.0	117.3	− 1
J.C.	119.0	117.6	− 1
A.G.	121.0	114.5	− 5

DISCUSSION

From Table I it is apparent from the indirect method, that below 10 cm. water pressure *order* only is expressed. From 10 to 15 cm. water, the variation (of one method to the other) is as much as 35 to 40 per cent. From 15 to 120 cm. water pressure, they may vary 20 per cent but never exceed that value. In general, the higher the values, the less the percentage variations of the two methods. While these variations do not necessarily represent the range of error, it appears that for the low values at least, the direct method is considerably more accurate than the indirect: from the standpoint of the direct method this accuracy depends upon its "objective" nature; low values are no more difficult to read than high ones. The "subjective" element present in the indirect technique plus the difficulty of determining the true end point for the low values, certainly suggests that the error is greater with the indirect method than with the direct when low pressures, i.e., below 15 cm. water, are being measured. If the low values are difficult to read with the indirect method, it is in most cases permissible to make the determination below heart level, that is, at a higher reading, and then correct. Even though variations between the two methods appear to be rather wide the accuracy obtained with either method, subject to the limitations described below, is as great as the circumstances warrant. This becomes apparent when the changeable nature of the venous pressure is considered. Alteration in the type of breathing, slight movements, slight strain and so on can produce great variations in the results.

The indirect method is much faster. It is possible, therefore, that part of the variation encountered between the two methods may be due to the fact that fluctuations in pressure are picked up by this method which are not detected by

the slower direct method. The estimated time required for a determination by the indirect method is of the order of two seconds as compared with not less than three or four minutes by the direct method; in other words, a pressure determined by the indirect method can be measured in $\frac{1}{100}$ of the time required by the direct method.

An experience with both methods is desirable if venous pressure is to be measured. If the indirect method is the one chosen for the problem to be undertaken, it is advisable at first for the beginner to check a few times the apparent end point of the indirect method with the direct determination in order to educate his eye.

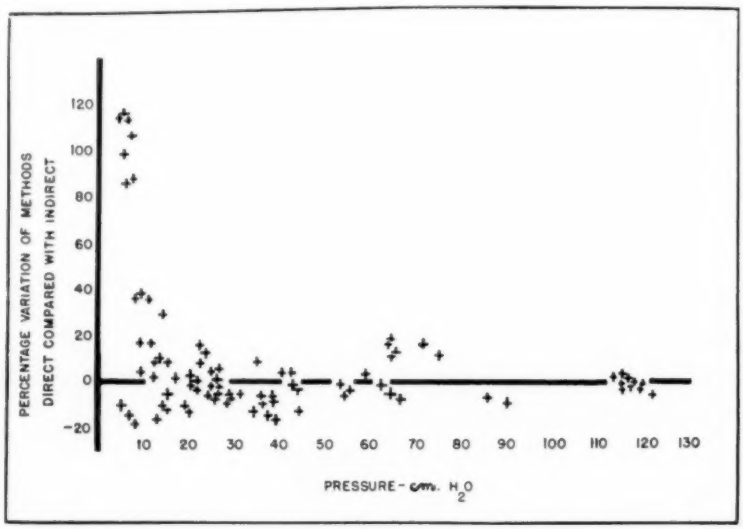

Fig. 1.—The percentage variation of the two methods showing large variations between them at values of venous pressure less than 20 cm. water, but at higher values no variations more than plus or minus 20 per cent. In general, the higher the venous pressure, the greater the correspondence.

ADVANTAGES OF EACH METHOD

Direct.—This method is more "objective," that is, the end point is easy to read. It has a greater precision than the indirect in the range below 15 cm. water pressure. The method can be used to determine the venous pressure in veins that are not visible, and hence it can be used over a relatively wide distribution of the venous system. Probably less experience is necessary for the successful use of this method than is the case with the indirect method.

Indirect.—The entire procedure can be simply and quickly carried out. There is no danger of sepsis, and the unpleasantness of the direct method is avoided; thus constriction of the vein studied and other circulatory changes are avoided. Adjustment of the zero point of the manometer to the capsule is not necessary. Once the capsule is applied, little mechanical difficulty occurs with the apparatus. The pressure in a given vein can be followed for hours; there is no time limitation here. A rapidly changing venous pressure can be accurately

followed. The method is as convenient for the determination of high pressures as for lower ones. Frequent determinations can be made without hesitancy; the patient remains cooperative.

DISADVANTAGES OF EACH METHOD

Direct.—Preparations and procedure are tedious. The sterile precautions necessary are time-consuming. There is at least the possibility of sepsis. To many subjects the venepuncture is very unpleasant. If frequent readings are wanted in order to follow the trend of venous pressure, repetition may be limited by this unpleasantness. Alterations of the circulation are occasionally produced by the psychic effect of the puncture as well as by the pain. On occasion, these effects are so extreme that fainting occurs; hence, infrequently it may not be possible to use this method. Puncture of the hand veins was followed in several cases by fainting. Starr and Collins[5] also reported this phenomenon. Hematomas occur at times and not only vitiate results but may be painful. Trauma of the vein is followed by such great constriction of it in some cases that the data obtained are of questionable value.[2] Careful adjustment of the zero point of the manometer is necessary. The determination of high pressures is inconvenient. The method is slow even after all preparations have been made. It cannot be used when the venous pressure is rapidly changing. The subject must remain motionless and not take any unusual breaths until the end point is established and checked. This may require so long a time that it is tiring to the patient. Use of the direct method was found impossible by one of us[2] in determining venous pressure at the ankle while walking. It is too slow to register the changes in pressure incident to the step when the method was used with its usual technique. This objection was overcome by employing a rigid Marey type of tambour, in which small volume changes registered great pressure changes. A second difficulty could not be overcome, i.e., the trauma incident to the insertion of an adequate cannula into the vein plus the unavoidable trauma which occurred during walking was enough to cause spasm and partial shutdown of the vein. This occurred even though the vein was blocked with procaine. What appeared to be moderate trauma was enough, in some cases, to give obviously incorrect venous pressure values. From this it was evident that the direct method for determining venous pressure has pitfalls that are great enough to make the method valueless under certain conditions. Considerable mechanical difficulty must be expected with this method, chiefly due to blood clots in the apparatus. The time allowed for a given series of determinations is distinctly limited, one-half hour is usually the upper limit, frequently much less than this time can be taken.

Indirect.—This method is "subjective," that is, the end point is sometimes difficult to read. Two observers are necessary, one to watch the vein, one the manometer. Only veins which are well exposed and which have a firm background are suitable for use. Some experience is necessary for the accurate use of the method. Below 10 cm. water pressure the method is inaccurate and "order" only can be determined at this low level.

SUMMARY

There are distinct fields of usefulness for each of the methods of determining venous pressure. There are conditions under which one method fails but not the other. The advantages and limitations of each method are pointed out.

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A METHOD FOR COLLECTING BLOOD FOR GAS ANALYSIS*

JOHN ADRIANI, M.D., NEW YORK, N. Y.

COLLECTING blood during surgical procedures that is to be analyzed for content of various gases presents difficulties. It is not possible to use the oil syringe or the oil flotation methods because the samples contain anesthetic agents readily soluble in hydrocarbons. The mercury displacement method frequently recommended, although the most satisfactory, is too cumbersome in the operating room. A method requiring little preparation which is simple in execution is needed. The method herein described permits the anaerobic collection and storage of blood, ready transference to analytical apparatus, and insures freedom from clotting.

The materials needed can be readily procured from the equipment ordinarily found in a hospital. A syringe, a three-way stopcock, a needle for venepuncture, a small amount of mercury, some saturated sodium oxalate solution, a large safety pin, and a piece of glass tubing are required. An ordinary 20 c.c. glass syringe with matched plunger and barrel, with a metal lock for fastening, is preferred. The tip must be ground perfectly to fit snugly into the adapter of the stopcock. The stopcock is the three-way metal Luer type, commonly used during encephalography and on pneumothorax apparatus, with a centerpiece

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which rotates easily but fits snugly. There must be no aspiration of air around the screw on the bottom which holds the centerpiece in place. This is the most probable source of air contamination and is difficult to avoid in old or worn stopcocks. A 20 gauge needle used for drawing blood for serologic and chemical analysis is employed. A piece of $\frac{3}{16}$ inch glass tubing, 2 inches long, is sealed at one end, slipped over the needle, sterilized, and fastened to the base of the needle with a speck of gummed paper to protect it until ready for use. As diagrammatically represented in the accompanying figure, a piece of rubber tubing (*E*), $\frac{3}{16}$ inches in diameter and $\frac{1}{4}$ inch long, is fitted over the junction of the needle and stopcock to make it airtight. It must not be long enough to

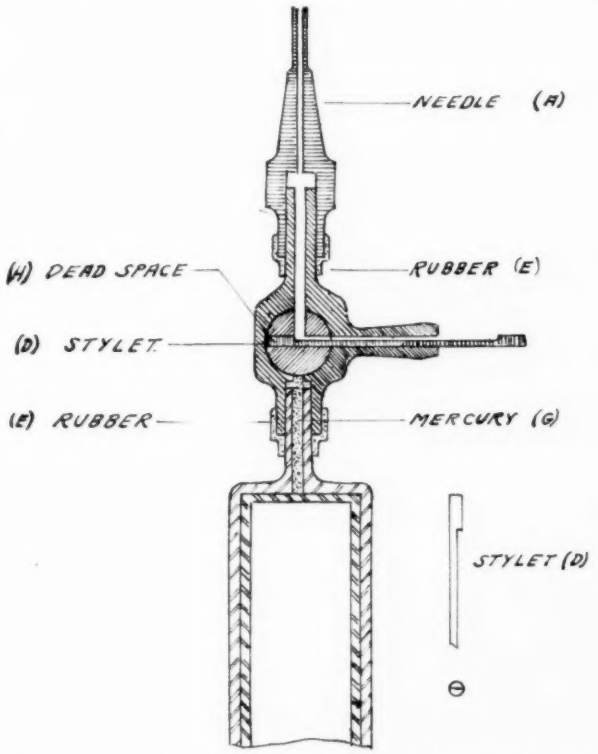

Fig. 1.—Diagrammatic section through needle, stopcock, and syringe. The needle at *A* connects to stopcock and is sealed with section of rubber tube, *E*. In a similar manner, syringe is sealed to stopcock with rubber, E_1. Mercury, *G*, fills the dead space in the syringe and stopcock. Stylet, *D*, is half-ground-down to the dead space, *H*, and projects from the sidearm.

push the needle outward. A similar piece (*E*), $\frac{1}{4}$ inch in diameter and $\frac{5}{16}$ inch long, is fitted over the junction of the syringe tip and stopcock. The dead space (*G*) in the tip of the syringe, which ordinarily contains a bubble of air measuring up to 0.5 c.c. is filled with mercury. This mercury is also used to agitate the blood with the anticoagulant and mix it when the formed elements have separated after the sample has been standing. The right-angle channel in the centerpiece of the stopcock, approximately 2 mm. in diameter, allows the shifting of flow from the side arm and the needle to the syringe and the needle. When the flow is from the needle to the side arm, there is a space (*H*) at the blind end of the channel which does not fill with blood and will pass as a bubble

of air, 2 mm. in diameter, or larger, when the flow is shifted to the syringe. To remove this bubble, a stylet (*D*) is made from the back of a large-sized safety pin which is slightly less in diameter than that of the channel. This is cut squarely at the end and made sufficiently long to fit into the side arm and protrude approximately ½ inch. Except for the part occupying the space to be obliterated, the pin is half-ground-down the entire length to form a channel for the blood and air, coming from the needle when the vein is first punctured. With this arrangement the syringe may be prepared several hours in advance, but should be cleaned if not used the same day so that the mobile parts will not become fixed.

In preparing the apparatus for use enough mercury is drawn through an unsterile needle into the barrel to form a layer ¼ inch deep. Sufficient saturated sodium oxalate solution is drawn up to overlay the mercury ½ inch when the syringe is inverted tip upward. Several cubic centimeters of air are next drawn in, and foam and air bubbles broken up. With the syringe tip upward air is first ejected, then the solution, and finally the excess mercury. Mercury will fill the space in the tip to the centerpiece after this excess has been forced through the side arm into the supply bottle. The stopcock is then turned so that the syringe is sealed leaving the needle connected with the side arm. Enough oxalate coats the inner glass and metallic surfaces to prevent a 15 c.c. blood sample from clotting. The outside of the stopcock is then rinsed with water to remove any oxalate, and a sterile needle fixed in place. Sterilization of the syringe and stopcock is not necessary. The stylet is fixed in position and the apparatus is ready for use.

Venepuncture is made and as soon as blood flows from the stylet it is withdrawn from the side arm and the stopcock turned so that the flow is into the syringe. When the desired amount of blood is obtained, the syringe is shaken gently for a few moments, and a few drops are forced through the side arm to remove the unoxalated blood in the tip and stopcock which would otherwise clot. The plunger is then locked and the syringe placed on ice until blood analysis can be done.

Syringes may have imperfect faces on the plungers so that a space is left in the center which does not entirely fill with mercury because of its surface tension. A very thin film of oxalate, amounting to less than 50 c.mm. will conveniently fill this space. The syringes should be selected with this in mind, particularly if the samples drawn are small.

AN IMPROVED METHOD FOR THE PREPARATION OF UREASE PAPER*

BERNARD KLEIN, M.A., BROOKLYN, N. Y.

IN THE routine determination of urea, both in blood and in urine, urease paper, because of its great stability and convenience has been a great aid. However, when the paper is prepared from the extract of Folin and Wu,[1] the strength of the paper necessarily depends upon the strength of the extract. Then too, the concentration of the enzyme is not constant in succeeding batches of jack bean meal, as it may be weakened by moisture, temperature, etc.

By incorporating the technique of Van Slyke and Cullen[3] with the method given by Hawk and Bergheim,[2] a uniformly high grade of urease paper has been obtained.

One part of jack bean meal (Arlington) is digested with 5 parts of distilled water at room temperature, with occasional shaking for one hour. The solution is cleared by centrifugation, and the supernatant liquid is poured with stirring into 10 volumes of acetone. The precipitate is filtered on a Buchner funnel and washed with a little cold acetone. The precipitate and paper is blown into a clean glass mortar and triturated with 10 parts of distilled water until a uniform suspension is obtained. The suspension is then poured into centrifuge tubes and centrifuged at moderate speed for fifteen to twenty minutes. The supernatant liquid is poured into a clean porcelain dish and at once taken up on strips of heavy filter paper and hung to dry over threads placed some distance apart, in a room free from air draughts and dust. When dry, the strips are at once cut into smaller strips, 1 cm. by 2.5 cm., and stored in a tightly stoppered wide-mouthed bottle. This urease paper keeps indefinitely and without loss of potency, and is best stored in a cool dry place.

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*From the Laboratories of the Beth-El Hospital, Brooklyn. Dr. Mendel Jacobi, Director.
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A FILTER ADAPTER FOR SMALL QUANTITIES OF SOLUTIONS

BOEN SWINNY, M.D., SAN ANTONIO, TEX.

THIS filter adapter is useful in the filtration of small amounts of solutions. For amounts larger than 20 c.c. obviously, the regular Seitz or Berkefeld filter is better.

Experimental work with saline washings of various cultures has shown that organisms cannot be cultured from the filtrate that has passed through this adapter. Ordinary saline solutions and blood serum are forced through with a 10 c.c. syringe easily in a few minutes with firm gentle pressure. Fifty per cent glycerin can be filtered, but requires considerable pressure.

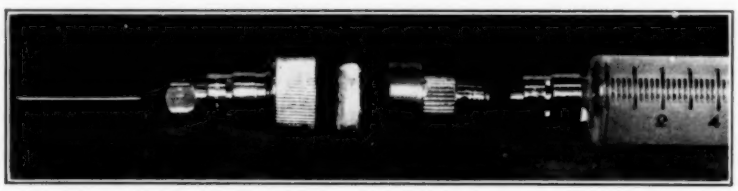

Fig. 1.—Seitz filter pad cut by one-half inch. Arch punch is used. A rubber washer is placed proximal and the small mesh wire screen is placed distal to the pad in the adapter (two-thirds actual size).

The advantages to be found in this new adapter are as follows:

1. There is no waste of filtrate as occurs with the use of the larger pads and candles. This is an important factor when one is working with rare and valuable extracts or solutions.

2. By passing the filtrate directly through an ordinary hypodermic needle into a sterile rubber capped vial, contamination is easily avoided.

3. Filtrates may be passed directly into dispensing vials, thereby saving time.

4. In doing passive transfers the serum may be drawn directly into a small hypodermic syringe and forced through the filter directly into the skin with an intradermal type needle. Thus filtration and sensitization of sites are performed at one step, saving time and avoiding contamination.

The author is indebted to Becton, Dickinson & Co., Rutherford, N. J., for assisting him in the construction of this instrument.

APPARATUS FOR ASEPTIC COLLECTION, DEFIBRINATION, AND DISTRIBUTION OF BLOOD*

L. H. Mardiss and F. T. Sokolik, Chicago, Ill.

WHEN large volumes of sterile blood are required for use in the laboratory, there is evident need of an apparatus designed to meet the essential requirements of asepsis and practicability. The apparatus to be described was finally evolved after testing many different devices. It has been in use in its present form for the past three years, and has given very satisfactory results. The basic apparatus is illustrated in the diagram and described as follows:

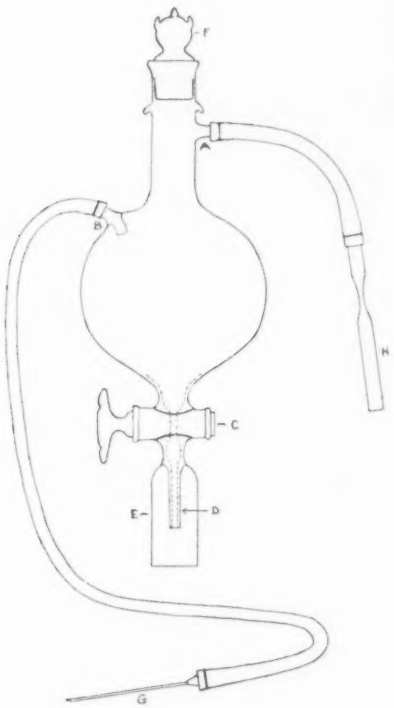

Fig. 1.

A round-bottomed flask, equipped with two side arms (*A* and *B*), is provided with an opening in its base. The opening is continuous through stopcock (*C*) and distributing tip (*D*). The distributing tip is inclosed by a bell shield (*E*). A ground glass stopper (*F*) fits into the ground neck of the flask. All parts are pyrex glass. Lower arm (*B*), fitted with rubber pressure tubing and hypodermic needle (*G*), conducts blood into the flask.

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Side arm (*A*) is connected to the glass tubing (*H*) by a short piece of rubber tubing. Reduced pressure is effected by attaching an aspirator pump to this air outlet line. Contained in the flask are copper wire rings which serve as defibrinating agents when set in motion. By manipulating the stopcock, blood may be distributed into sterile containers. During this part of the procedure the bell shield provides protection against contamination.

Preparation of the Apparatus.—To maintain sterility of the apparatus after sterilization, it is essential that all connections are airtight. For effective seal at the ground surfaces of the stopper and stopcock plug, heavy grease must be applied very carefully. The stopper and stopcock plug are held firmly in place by rubber bands. All rubber tubing connections are bound with heavy iron wire. Nonabsorbent cotton is packed into the glass tubing of the air outlet line. The hypodermic needle is attached and securely wired to the rubber tubing of the blood inlet line. It is inclosed in a test tube which extends a distance over the rubber tubing. A cotton strip, about 4 inches wide, is wrapped around the upper part of the protective test tube and the adjoining rubber tubing. The cotton may be securely held in place by strings or rubber bands. This wrapping aids to protect the sterility of the hypodermic needle. It also holds the test tube firmly in place.

The bell shield is packed with a nonabsorbent cotton plug. This shield with its contained plug is wrapped with heavy paper. Defibrinating rings are formed from copper wire. They are of such size as to pass easily through the neck of the flask. Twenty to twenty-five rings effectively defibrinate an 800 c.c. volume of blood. Various anticoagulants may be substituted for the defibrinating rings. The apparatus is sterilized for subsequent use by autoclaving at 15 to 20 pounds' pressure for thirty minutes.

Collection and Defibrination.—After the skin site of the donor has been shaved and properly disinfected, the vein is pierced by the needle. When blood appears in the collecting vessel, the attached aspirator pump is started and adjusted to a reduced pressure reading of about ½ pound. The flask is then moved in a circular manner in a horizontal plane setting the defibrinating rings in motion through the blood. Defibrination should continue for at least five minutes after collection has been stopped. During the entire period care should be taken to avoid splashing blood into the air line.

After a sufficient volume of blood has been collected, the blood line is tightly closed off with a screw clamp and the needle is withdrawn from the vein. Preliminary closing of the blood inlet line (before the needle is withdrawn) will obviously prevent contaminating air from being drawn into the apparatus.

Distribution.—The apparatus is secured to a ring stand placed in a glass hood. Each piece of auxiliary equipment has been previously swabbed with mercuric chloride to reduce the possibility of contamination. With the wrapper and cotton plug removed from the bell shield, it is flamed, and the blood is distributed into sterile containers by manipulating the stopcock. During the operation the bell shield is flamed frequently.

Cleaning the Apparatus.—The hypodermic needle is detached and cleaned separately. Fibrin is removed from the wire rings by brushing them under the tap. Overnight infusion in trypsin solution very effectively dissolves all fibrin. The remaining apparatus is readily cleaned by the use of cold water which is run through its various parts. The apparatus is now ready for reassembly.

THE DETERMINATION OF SULFANILAMIDE IN BLOOD*

JONAS KAMLET, BROOKLYN, N. Y.

MARSHALL, Emerson, and Cutting have described a method for the determination of sulfanilamide in blood which comprises diazotizing the alcoholic blood filtrate and coupling with dimethyl-∝-naphthylamine. The resultant azo compound, 1-(p-sulfamido-phenylazo)-4-dimethylnaphthylamine, is deep red in color and can be detected in a dilution of one part in ten million of water.

From the practical point of view, however, the diazotization of an alcoholic blood filtrate is undesirable. On dilution and acidification, the alcoholic filtrate becomes very turbid due to the precipitation of lipids and cholesterol, thus rendering colorimetric comparison impossible. It is often necessary to filter the turbid solution two or three times to obtain a filtrate sufficiently clear for comparison. A series of experiments was, therefore, conducted to adapt this specific and sensitive color reaction to other blood filtrates.

A 0.1 per cent solution of sulfanilamide in physiologic saline was added to normal blood, so that concentrations of 5.0 to 35.0 mg. per cent of sulfanilamide resulted. With the use of 5 per cent trichloracetic acid as a precipitant, the recovery of sulfanilamide in the filtrate ranged from 96.5 per cent to 102.0 per cent, with an average of 99.2 per cent. The troublesome turbidity of the Marshall method is obviated, and clear solutions suitable for direct comparison are obtained:

REAGENTS REQUIRED

1. Trichloracetic acid, 5 per cent solution. Dissolve 50 gm. (C.P.) trichloracetic acid in 800 c.c. of water and dilute to 1 liter.

2. Sodium nitrite, 1 per cent solution. Dissolve 10 gm. (C.P.) sodium nitrite in 800 c.c. distilled water and dilute to 1 liter. This solution will keep indefinitely in an amber-colored, glass-stoppered bottle.

3. Dimethyl-∝-naphthylamine, 1 per cent solution. Dissolve 1 c.c. of this compound (Eastman Kodak) in 100 c.c. of 95 per cent alcohol.

4. Ethyl alcohol, 95 per cent.

*From the Department of Laboratories, Israel Zion Hospital, Brooklyn.
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5. Sulfanilamide standard solution. Dissolve exactly 200 mg. of C.P. sulfanilamide in 800 c.c. of boiling distilled water, let cool to room temperature, and dilute to 1 liter. One cubic centimeter of this solution contains 0.2 mg. of sulfanilamide.

PROCEDURE

To 2 c.c. of oxalated or citrated whole blood, add 18 c.c. of 5 per cent trichloracetic acid solution, mix, let stand for ten minutes, and then filter through a retentive, ashless filter paper.

Pipette 10 c.c. of the filtrate into a 6 by 1 inch test tube. To a similar tube, add 1 c.c. of sulfanilamide standard, 6 c.c. of trichloracetic acid solution, and 3 c.c. of 95 per cent alcohol. To both add now 0.1 c.c. of 1 per cent sodium nitrite solution, mix and let stand for three minutes.

One cubic centimeter of the dimethylnaphthylamine solution is now added to each tube with gentle shaking, the tubes are set aside for ten minutes and then compared in the colorimeter.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 20 = \text{mg. sulfanilamide per 100 c.c. whole blood}$$

Set the unknown at 20.0 mm. and compare. Reading of standard equals milligrams of sulfanilamide per 100 c.c. whole blood.

A series of 50 comparative blood determinations on patients being treated by peroral administration of sulfanilamide was made, using the Marshall method as a basis of comparison. Bloods containing from 2.5 to 18.5 mg. per cent of sulfanilamide were encountered. The maximum deviations of the above-described method from the Marshall method were minus 4.5 per cent and plus 3.3 per cent, with an average agreement of 98.8 per cent.

REFERENCE

Marshall, E. K., Jr., Emerson, K., and Cutting, W. C.: Para-Aminobenzenesulphonamide—Method of Determination in Urine and Blood, J. A. M. A. **108**: 953, 1937.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

FLUORIDE POISONING, Composition of Human Bone in Chronic, Wolff, W. A., and Kerr, E. G. Am. J. M. Sc. **195**: 493, 1938.

Human bones from a case of chronic fluoride poisoning contained normal percentages of calcium, phosphorus, and carbon dioxide. The fluorine content was increased up to twenty fold the normal value and unequally distributed in various parts of the skeleton, highest in the vertebrae and lowest in the long bones. The specific gravity of the femur was low. Data on man and animals are compared.

HYPERGLYCEMIA Without Glycosuria, Davidson, C. F. Endocrinology **22**: 493, 1938.

Hyperglycemia without glycosuria has been revealed by means of glucose tolerance testing in an amazingly high percentage of cases, in which the author's judgment has deemed a study of endocrine balance to be indicated.

ERYTHROCYTES, Staining of, in Urinary Sediment, Endtz, W. A. München. med. Wchnschr. **85**: 141, 1938.

There are two stock solutions:

The first consists of 1 part of benzidine in 100 parts of diluted alcohol; the second consists of 20 parts of a 6 per cent solution of acetic acid in 100 parts of distilled water. The reagent that is used for staining the erythrocytes is composed of 1 c.c. of the first solution, 2 c.c. of the second solution, 5 drops of a 3 per cent solution of hydrogen peroxide, and 9 c.c. of distilled water. Following centrifugation of the urine, the fluid is carefully poured off so that as little as possible remains in the sediment. Then a drop of the reagent is put into the centrifuge tube and, by slight tapping, is mixed with the sediment. If a urine contains much sediment, it is advisable to place a drop of it on a slide and then add a drop of the reagent. The two drops are mixed by drawing the cover glass in the oblique position back and forth over the slide. Inspection of the stained preparation reveals that all formed elements of the blood have turned blue, whereas yeast cells and all other elements not belonging to the blood remain unstained. The erythrocytes show a uniform blue with only a few isolated granules. The leucocytes show a nuclear blue stain; the protoplasm contains many blue granules. These granules may be so numerous and their coloring so intense that the nuclear stain may disappear among them. The large lymphocytes can be recognized by their round nuclei and by their protoplasm with many granules. The small lymphocytes are differentiable from the erythrocytes by their large number of granules. If a more distinct differentiation of the erythrocytes and leucocytes and of the various types of leucocytes is desired, it is advisable to use a reagent with a higher acetic acid content. The author recommends the following reagent: 0.5 c.c. of concentrated solution of benzidine (as indicated), 3 drops of a 3 per cent solution of hydrogen peroxide, 5 drops of distilled water, and 5.5 c.c. of a 1 per cent solution of acetic acid. This reagent is best for the differentiation of the different types of blood corpuscles, but, if only erythrocytes are looked for in the sediment, the first mixture is the best.

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TRANSFUSION, Inexhaustible Supply of Blood for, and Its Preservation. Goodall, J. R., Anderson, F. O., Altimas, G. T., and MacPhail, F. L. Surg., Gynec. & Obst. 66: 176, 1938.

The authors use placental blood collected and preserved as described below:

When the baby is born, it is laid upon the mother's abdomen. The cord is tied or clamped and wiped clean with a sponge moistened in 75 per cent alcohol. The cord is stripped free of blood for about 6 inches, by the left finger and thumb armed with a sponge. The cord is cut with a sterile scissors, the severed end lowered to below the vulva. A special towel with a 2 inch hole in its middle is put over the hand, holding the cord in such a manner that the hand is opposite the hole. The towel is clamped to the operator's gown near the elbow with an Allis forceps. The end of the cord is passed through the aperture in the towel, and so placed that its end hangs in the funnel of the receptacle held by the nurse. Pressure on the cord is then released completely and the blood is collected. Pressure on the fundus by the nurse or assistant hastens the emptying of the placenta. When completed, the cord is clamped and the collecting has been accomplished. The towel described is used to prevent any contamination of the cord and receptacle by any fluid extraneous to that under collection. The process occupies about three minutes, and the average amount collected from each patient may vary from 100 to 150 c.c. The technique used in collecting the blood is extremely simple, and blood is always available when it is needed for transfusion.

Preservative: The preservative that has been found to be most suitable, that proposed by the Moscow Institute of Hematology, consists of the following ingredients and their dilutions:

Sodium chloride	7.0 gm.
Sodium citrate	5.0 gm.
Potassium chloride	0.2 gm.
Magnesium sulfate	0.004 gm.
Bi-distilled water	1000 c.c.

This solution is now put up in ampoules of 25 c.c. which, when added to 100 c.c. of distilled water, gives one the proper dilution, so that preservative and blood from each case are mixed in equal proportions, 125 c.c. of preservative and, roughly, 125 c.c. of fetal blood.

Preparation of receptacle: A wide-based glass flask of 300 c.c. capacity and a glass funnel are thoroughly cleansed and rinsed several times with distilled water. One hundred cubic centimeters of distilled water and the contents of an ampoule are put into each flask and corked with a stopper of absorbent cotton in gauze tied at the top. The flask and funnel are wrapped separately in a cloth and sterilized in the autoclave. When cooled, they are ready for use. When blood is to be collected, the funnel is partially released from its cover and is handed aseptically to the surgeon. The flask covering is released from the top, so that when the nurse holds the flask by the base the cloth falls over her hand. The surgeon places the funnel in the flask, and the nurse holds these in position for receiving the blood. When the blood has been collected in the flask, a few drops from the end of the cord are taken in test tubes, one for grouping, the other for the Wassermann, if the mother's blood has not already been tested for the reaction. As all prospective mothers in our service have had Wassermann reaction tests made during their pregnancies, duplication of this test is not necessary. The flask and tubes are labeled with the patient's name and the date and are transferred to a refrigerator with a temperature between 33° and 38° F. Temporary changes of this temperature do not affect the character of the blood.

The blood is ready for transfusion as soon as the grouping and reaction are completed. Groups 2 and 3 are kept in the upper shelves of the refrigerator. Groups 1 and 4 on the lower shelves, so that at a glance one can estimate the quantities in stock.

A clock thermometer on the top of the refrigerator indicates the temperature so that it is not necessary to open the door. Defrosting should be done once a month, without removing the blood.

Conditions: There are certain conditions which become axiomatic as regards mother, child, and accidents of labor. Blood of the placenta will, of course, not be taken in cases of obvious transmissible disease in either mother or child. Eclampsia is not a contraindication. Blood will not be taken, however, in cases in which the membranes have ruptured for more than forty-eight hours before delivery, or in cases of definite prematurity. In cases of marked asphyxia pallida, the amount of blood in the placenta is so small as to make it worthless.

Observations: Babies at full term have a fixed blood group. Consequently, the groups of donors of placental blood are relatively numerically the same as in the recipients. The supply in each group, therefore, will approximate the demand for each group. Major reactions are all that are necessary in crossmatching. Cultures of the preserved blood are unnecessary because, at the low temperature for preservation, contamination, if present, could not propagate, and consequently would be so attenuated as to be innocuous. When the placental blood settles, it frequently differentiates itself into three layers. The lowest is composed of red blood cells, the next is a thick buffy coat, and the third the supernatant clear fluid. Slight progressive hemolysis and consequent coloring of the supernatant fluid may occur after the first fifteen days. This, even major degrees of hemolysis, is no barrier to a normal transfusion. Stirring or unnecessary movement of the blood is productive of slight hemolysis. The reason for the innocuousness of this hemolysis lies in the fact that it would seem that the hemolysin is in infinitesimally small quantity and is inoperative in the recipient. Moreover, it is believed that the hemolysis is the result of the preservative upon fetal nucleated red blood cells, of which normal fetal blood contains quite a few, which possess greater fragility than adult red cells.

TISSUE: Rapid and Economical Method for Staining Routine Tissue Sections With Hematoxylin and Eosin. Arch. Path. 25: 376, 1938.

The principle of the modification consists in the dehydration of the section before counterstaining and the application of eosin as a solution in carbolxylene. Then, since the tissue passes afterward through xylene only, there is no opportunity for decolorization. Alcohol is saved because dehydration can be commenced in the stronger alcohol, which obviates the use of the lower gradations. The solution of eosin in carbolxylene, which constitutes the principal element of modification, may be called for convenience eosinol.

Preparation of Eosinol.—Dissolve 5 gm. of aqueous eosin in 10 c.c. of distilled water. Precipitate it by adding 10 c.c. of glacial acetic acid and mix with a glass rod. Incubate the resulting coagulum at 56° C. for from twelve to sixteen hours, or until all the water has evaporated.

Dissolve this dehydrated acid-eosin in 10 c.c. of absolute alcohol and 20 c.c. of acetone, stirring with a glass rod for several minutes. Let the undissolved portion, which is to be discarded, settle to the bottom of the container (about ten minutes). Remove the clear portion with a clean dry pipette; add it to 1,500 c.c. of carbolxylene (1 part pure phenol crystals in 3 parts of neutral xylene). Some precipitate will form, which will settle to the bottom of the container. The clear portion is eosinol. The solution keeps indefinitely.

Owing to variation in the staining power of the various brands of powdered eosin, it may be necessary to standardize the solution by staining control sections and adjusting the strength of the eosinol by reducing or increasing the amount of carbolxylene.

Staining Method.—1. Prepare microscopic sections by one of the standard sectioning methods (i.e., employing frozen tissue or tissue embedded in paraffin or pyroxylin) and stain in alum-hematoxylin (Harris, Delafield) for from three to seven minutes.

2. Wash in tap water until blue (about thirty seconds).

3. Destain in acid-alcohol (1 c.c. of concentrated hydrochloric acid in 99 c.c. of 70 per cent alcohol) by dipping the section in and out for even destaining.

4. Rinse in tap water.

5. Blue by dipping in 2 per cent ammonia water (2 c.c. of strong ammonium hydrate in 98 c.c. of tap water).

6. Wash in tap water for a few seconds.

7. Wipe off the water with a towel, dehydrate in 95 per cent alcohol for one minute, and wipe off the back of the slide.

8. Dehydrate completely by pouring on a few drops of absolute alcohol from a drop bottle. Repeat.

9. Counterstain and partially clear in eosinol for from ten to thirty seconds, depending on the strength of the solution.

10. Treat with carbolxylene for three minutes.

11. Treat with first xylene two minutes.

12. Treat with second xylene two minutes.

13. Treat with third xylene two minutes.

14. Mount in gum dammar (saturated solution of gum dammar in neutral histologic xylene prepared by the Eastman Kodak Co.).

PNEUMONIA. Study of a Type I Epidemic at the State Hospital at Worcester, Massachusetts, Smillie, W. G., Warnock, G. H., and White, H. J. Am. J. Pub. Health 28: 293, 1938.

An extensive epidemic of lobar pneumonia in 1937 at the State Hospital in Worcester, Mass., which began during an influenza outbreak, was due to the Type I pneumococcus.

Type I pneumococcus carriers became widely distributed throughout the Main Hospital. These carriers, and not the actual cases of pneumonia, were responsible for the spread of the infection.

The epidemic at the Main Hospital ceased abruptly following administration of Type I soluble antigenic substance (Felton antigen) to the hospital population.

A fresh outbreak of Type I lobar pneumonia occurred at Summer Street Division (a branch hospital) one week after the epidemic at the Main Hospital abated. This infection entered Summer Street Division through normal carriers of Type I pneumococcus, who were transferred from the Main Hospital to the subsidiary hospital. The administration of antigen at Summer Street was also followed by immediate cessation of this outbreak.

RABIES, Comparative Methods of Diagnosis of, in Animals, Leach, C. N. Am. J. Pub. Health Suppl. 28: 162, 1938.

A technique for the earlier diagnosis of rabies by mouse inoculation is described. In addition to shortening the period required for animal diagnosis, the method has the advantage of considerably reducing the cost. Comparative results from brain material of 1,032 animals, diagnosed by the usual methods in several laboratories and then injected into mice, confirmed the positive results obtained by the usual methods, but disclosed an error of 12.0 per cent for the brains reported negative for Negri bodies.

The vaccination or treatment of animals with rabies vaccine apparently increases the difficulty of diagnosis by the discovery of Negri bodies.

Four- to six-week-old white mice are highly susceptible to the rabies virus when inoculated intracerebrally with brain emulsion from a rabid animal.

Material, when not examined fresh, is preserved in full strength glycerin. A small portion of the Ammon's horn is ground up in a sterile mortar and 9 parts of hormone broth added to 1 part of ground brain material. This is thoroughly emulsified and then centrifuged at 2,000 to 2,500 revolutions per minute for about five minutes. With a 0.25 c.c. tuberculin syringe, 0.03 c.c. of the supernatant fluid is injected into the brain through the skull of a four- to six-week-old mouse. A 27 gauge needle, ¼ inch long, is employed, and the injection made slightly to one side of the midline of the skull and half way between the eye and ear. The site of inoculation is moistened with alcohol before injecting. The mice are lightly

etherized before the operation. It has been the practice to inoculate four mice with each brain specimen. When the injections are performed by experienced technicians, one can expect a mortality of 0.5 to 1.0 per cent due to mechanical injury.

When the disease appears among a group of inoculated mice, in the great majority of instances all mice in the group succumb. One survivor has been observed from each of 4 groups, and in 3 groups there were 2 survivors in each of 4 inoculated. The survivors may show early symptoms and later recover, but no recoveries are recorded after the disease reaches the paralytic stage.

After the development of paralysis, death follows on an average of approximately forty-eight hours. Sluggishness, roughening of the fur, and loss of hair luster appear as the earliest symptoms, followed by photophobia in many cases and a conjunctivitis with seropurulent discharge. Paralysis, most frequently of the hind legs, appears, and the legs are extended either to the sides or straight back. At this stage convulsions are frequently seen, especially when the animal is disturbed by jarring or noise. Death is preceded by complete prostration and labored and irregular respiration. Of the 2,095 mice injected with rabies positive brain material, the majority died in from ten to twelve days.

MILK, Present Status of Phosphatase Test for Pasteurization, Tiedeman, W. V. D. Am. J. Pub. Health **28**: 316, 1938.

The test is based upon the action of the enzyme phosphatase which is present in raw milk but is almost completely devitalized by pasteurization on a buffer substrate containing disodium phenol phosphate, Folin's reagent also being added. During incubation for from eighteen to twenty-four hours at 37° C., phenol is liberated in proportion to the amount of phosphatase present which after clarification of the sample is read by comparing the intensity of the blue color in test tubes with that produced by known concentrations of phenol or with permanent standards. Controls are used.

Experience in the practical use of the phosphatase test for pasteurization convinces the author that it has great practical value. Although continued use may reveal interfering substances, no serious interference has been encountered to date. Those health officials who recognize the importance of pasteurization of milk to public health and their responsibility to the public to see that milk and cream so labeled are actually pasteurized, will welcome this test.

CARCINOMA of the Breast, Value of Preoperative and Postoperative Irradiation, Pfahler, G. E., and Vastine, J. H. J. A. M. A. **110**: 544, 1938.

Reviews of records from clinics in different parts of the world and a postoperative study of 400 cases of carcinoma of the breast belonging to clinical stage 2 treated more than five years ago in which operation took place more than five years ago, convince the authors that postoperative prophylactic irradiation is of definite value.

Such postoperative treatment should begin, when practical, within ten days to two weeks after operation.

Almost every physician has seen recurrent carcinoma of the breast disappear after irradiation. It should be self-evident that similar treatment given to the cancer cells before the disease has adapted itself to the host and has become macroscopic should accomplish even better results.

Preoperative irradiation is probably of equal or greater importance. It should be employed within approximately two weeks before operation in cases of carcinoma of the breast in clinical stage 2. Doubtfully operable carcinoma and carcinoma in stage 3 are treated over a much longer time.

The preoperative treatment is intended to devitalize the more malignant cells, which usually cause the surgical failures.

Statistics are not yet available, but the authors believe that skillful preoperative irradiation, then skillful operation, followed by skillful postoperative irradiation should double the total number of persons with cancer of the breast remaining well five years.

Statistics compiled from the same clinics in which both forms of treatment were used show an improvement of from 11 per cent to 73 per cent when postoperative irradiation is combined with operation as compared with operation alone, according to different authors. Large collections of cases from literature show a 25 per cent improvement.

The average number of persons with carcinoma in stage 2 who survived when treated by operation alone, for a group of the best surgical clinics, is 28 per cent, while the general average for postoperative irradiation is 40 per cent. The authors' results with postoperative irradiation of carcinoma in stage 2 show 52 per cent, and when the cases in which preoperative irradiation was used are added, it is 57 per cent.

GRANULOMA VENEREUM, Pathology of, D'Aunoy, R., and Von Haam, E. Am. J. Path. **14**: 39, 1938.

The pathology of granuloma inguinale has been studied in a series of 294 cases observed over five years at the State Charity Hospital of Louisiana at New Orleans.

The typical manifestations of the disease embrace nodular lesions and serpiginous ulcerations, which have a tendency to spread along the moist folds of the pudendal region, healing with the formation of atrophic scars.

Atypical manifestations are produced by unexplained increased fibroblastic reaction of the host leading to hypertrophic and cicatricial (keloid-like) lesions, which must be considered active stages of the infection.

Secondary infection produces serious ulcerative necrotic lesions which may severely mutilate the infected parts and give rise to sepsis and toxemia.

Histopathologic study of biopsy material and tissue obtained at autopsy reveals that the stage of infiltration is quickly followed by the stage of granulation, during which the epithelial lining of the skin or mucous membrane is perforated by a vascular granulation tissue. Donovan organisms can be demonstrated in the infected tissue during all stages of the infection.

Search for a causal agent has resulted in the isolation of an organism belonging to the Klebsiella group. Inoculation of various laboratory animals with this organism has failed to incite lesions comparable to the disease in the human being, although such lesions have been reported as produced by inoculation of material derived from human cases.

PATCH TESTS, A New Method of Making Multiple, Biederman, J. B. Arch. Dermat. & Syph. **37**: 198, 1938.

One strip of wax paper, about ½ to ¾ inch (1.2 to 1.9 cm.) wide, is placed along the length of the forearm. One end of the wax paper is fastened above the wrist with a strip of adhesive tape from ½ to 1 inch (1.2 to 2.5 cm.) wide. With an indelible skin pencil a numeral is placed on the skin. Immediately adjacent to this numeral is placed a small square of white blotting paper impregnated with the substance to be tested. A narrow strip of adhesive tape is then placed on the forearm above the tested substance to limit the motion of the small square of white blotting paper. The numeral "2" is written immediately above this last piece of adhesive tape, and the second substance to be tested is placed adjacent to this number. This procedure is repeated until from twelve to twenty patches are placed on each forearm.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Introduction to Ophthalmology*

THE purpose of this book is to present clearly, simply, and in a readily assimilable manner that part of ophthalmology forming an essential part of basic medical education. The text, therefore, is concerned primarily with the principles of ophthalmology and the pathogenesis of diseases of the eye, and does not touch upon methods of examination, diagnosis, or treatment. Individual diseases are discussed as they illustrate pathogenetic principles.

The book is well planned and well written and admirably fulfills its purpose. Although addressed primarily, perhaps, to the medical student, the physician may read it with profit, and it can be recommended as a useful addition to the medical library.

Middle Age Is What You Make It†

WHILE this book is addressed primarily to the laity, it can be read with interest by the physician who will find in it answers to some of the problems brought to him as well as the queries of that great group numbered among "the middle aged."

The character of the book is evidenced by its chapter headings: Happiness and Health; A Middle Aged Man Should Have His Own System of Living; The Friendly Microbe; "Acid Condition"; Self-Medication and Constipation; The "Why" of Nervous Indigestion; Age Discrimination and Chronic Disease; Immortality and Old Age; The Defects of Our Mechanism; Vitality and Fatigue; Old Truths Still Stand; Enjoy Your Middle Age.

The individual apt to be unduly impressed with the more or less pseudoscientific barrage emanating from radio programs and exploding in full-page advertisements—in each instance frequently inaugurated by the solemn phrase, "a group of scientific investigators have found," etc. —can read this book with profit.

And having done so, he will without doubt have a clearer understanding of vitamins, diet, "acid conditions," and so on, than can be derived from the "information" ladled out for him between radio bands and radio "comics"—so-called. It is even possible that he may appreciate that middle age, though inevitable, is not per se a catastrophe but—within reason—whatever he may make of it himself.

The writing of popular books on medical subjects is by no means a simple job. Dr. Sokoloff has managed to compress a great deal of common sense into relatively few easily readable pages.

*Introduction to Ophthalmology. By Peter C. Kronfeld, M.D., Professor of Ophthalmology, Peiping Union Medical College. Cloth, 331 pages, 32 illustrations, 5 plates. $3.50. Charles C. Thomas, Springfield, Ill.

†Middle Age Is What You Make It. By Boris Sokoloff, M.D. Cloth, 201 pages. $1.75. The Greystone Press, New York, N. Y.

Essentials of Psychiatry*

EVEN a cursory perusal of this book suffices to indicate why it has reached a third edition and why it is a deservedly popular text.

The physician of today must have some understanding of psychiatry. This is no longer debatable. Fortunately, in this book he may readily acquire a sufficient grasp of this study to enable him to understand those patients—by no means a small group—whose mental aspects may definitely influence the conditions which lead them to consult the physician.

As Dr. Campbell says in his introduction: "The student or practitioner receives from this book an excellent orientation in psychiatry and will find in it a sound preparation for more intensive study."

This book may well have a place in the library of every physician.

Textbook of Clinical Pathology†

PERHAPS the most difficult problem in the writing of a book is that of determining its character and contents. For a text intended for the specialist must necessarily differ from that intended for the beginner. If it is determined to walk a middle path and to attempt to serve both extremes, the problem becomes doubly difficult and emphasizes the truth of the axiom concerned with carrying water on both shoulders.

In its preface this book is said to be directed to the medical student and physician. This is borne out by its opening chapter. But as one goes from chapter to chapter there are found many procedures which the physician has neither the equipment nor the training and skill to apply with satisfaction. On the other hand, the clinical pathologist misses many with which he is daily concerned.

The intentions of the authors (for this is a book written by twelve collaborators) are obvious. But while their purpose is evident, they have had difficulty with its achievement. As an interpretative text it is hardly sufficiently comprehensive for the physician; as a technical manual it includes much that he will seldom, if ever, attempt. And, as has been said, the pathologist and laboratory worker will find it somewhat limited for their purposes.

While it will hardly serve as an exclusive text for either group, both may find it of value as a supplementary volume for their shelves.

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*Essentials of Psychiatry. By George W. Henry, M.D., Associate Professor of Psychiatry, Cornell University Medical School, With an Introduction by Charles Macfie Campbell, M.D., Professor of Psychiatry, Harvard University. Cloth, ed. 3, 465 pages. $5.00. Williams & Wilkins Co., Baltimore, Md.

†Textbook of Clinical Pathology. Edited by R. R. Kracke. Cloth, 567 pages, 205 figures, 31 colored plates. $6.00. Williams & Wilkins Co., Baltimore, Md.